2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of heptachlor and heptachlor epoxide and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for heptachlor and heptachlor epoxide based on toxicological studies and epidemiological investigations.

There are few studies that specifically describe the effects of heptachlor or heptachlor epoxide in humans following exposure via the oral, inhalation, or dermal routes. There are data on the health effects of chlordane from occupational studies of pesticide applicators and manufacturers, and from studies of people who consumed food contaminated with chlordane and heptachlor. Chlordane is a pesticide that is structurally similar to heptachlor, and technical-grade preparations may contain anywhere from 6% to 30% heptachlor. While the effects of two such structurally similar compounds would be expected to be essentially similar, there might not be a one-to-one correspondence of effects, and data do not exist with which to compare the toxicities. The q_1* is the numeric value that is used to provide an estimation of the carcinogenic potency of a chemical. The EPA q_l* for chlordane is lower than that for heptachlor. The q_l* for heptachlor is lower than that for heptachlor epoxide. Based on general toxicity data in laboratory animals, heptachlor would appear to be more toxic than chlordane. Heptachlor epoxide is more toxic than heptachlor.

In addition to being a component of technical-grade chlordane, heptachlor is a metabolite of chlordane. Therefore, identification of heptachlor or heptachlor epoxide does not always signify that the primary exposure was to heptachlor. Humans have been exposed occupationally to heptachlor via the inhalation and dermal routes during manufacture and application of pesticides. The general population has been exposed through the inhalation or dermal routes following the use of chlordane or heptachlor in homes, and orally through the consumption of contaminated food. Much of the human data on exposure to heptachlor is limited because of concomitant exposure to other substances. Toxicological and pharmacological animal studies have tested heptachlor primarily by the oral route of exposure. The existing animal studies share similar limitations. For example, the National Cancer Institute (NCI) bioassay on the effects of heptachlor was carried out with a formulation of technical-grade heptachlor that contained 22% α-chlordane. Chlordane, heptachlor, and heptachlor epoxide have been classified as B2 carcinogens, or possible human carcinogens (IRIS 1990).

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure--inhalation, oral, and dermaI-- and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods—acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should

also help to determine whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with the carcinogenic effects of heptachlor and heptachlor epoxide are indicated in Figure 2-l. Because cancer effects could'occur at lower exposure levels, the figures also show a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability and extrapolation of data from laboratory animals to humans.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, the MRLs may be derived.

2..2.1 Inhalation Exposure

2.2.1 .l Death

A retrospective mortality study conducted on 1,403 white male workers engaged for at least 3 months in the manufacture of chlordane, heptachlor, and endrin between 1946 and 1976 showed a statistically significant increase ($p<0.05$) in deaths due to cerebrovascular disease when compared to U.S. mortality data (Wang and MacMahon 1979b). No clear relationship with employment duration, duration of follow-up, or age was found. An attempt was made to examine the relationship between exposure intensity and mortality, but complete occupational histories were not available in all cases. The study is also limited by lack of quantitative exposure information, concomitant exposure to other chemicals such as aldrin, endrin, and diazinon, and lack of control measures for confounding factors such as smoking. A larger occupational cohort of male pesticide applicators followed prospectively showed that for 16,124 workers employed for 3 months or more between 1967 and 1976, the standardized mortality ratio (SMR) was less than 100, indicating that there was no increase over expected deaths due to all causes (MacMahon et al. 1988; Wang and MacMahon 1979a). Specific dose and exposure information was not provided. These occupational studies are presumed to reflect primarily inhalation exposure, with some concomitant dermal exposure. Mortality due to bladder cancer was on the border of statistical significance. The results of the study suggested that blood pressure and cerebrovascular disease were end points to follow closely.

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A mortality study of a cohort of 3,827 licensed male pesticide applicators was conducted in Florida. This cohort did not exhibit the healthy worker effect, as the overall SMR was close to expected (Blair et al. 1983). Increased SMRs, although not statistically significant, were seen for leukemia, cancers of the brain, and lung cancer. Follow-up was achieved for over 95% of the identified cohort members, but no information was available for smoking history.

No studies were located regarding death in animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.2 Systemic Effects

No studies were located regarding respiratory, gastrointestinal, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide.

Cardiovascular Effects. The information regarding cardiovascular effects in humans associated with heptachlor and heptachlor epoxide exposure is limited to a case report (Pines et al. 1986). Sixty-two hospital patients with no known occupational exposure to pesticides were divided into three groups: Group A comprised 8 men and 3 women with mild to moderate arteriosclerosis; Group B comprised 18 men and 6 women with moderate to severe arteriosclerosis; and Group C comprised 19 men and 8 women without obvious signs of arteriosclerosis and served as the study control. Several organochlorine compounds, including heptachlor epoxide, were determined in the patients' blood serum. Groups A and B had higher heptachlor epoxide blood levels (7.5 and 8.0 ng/g serum, respectively) than Group C (6.5 ng/g serum). The elevation in Group B was statistically significant. This report cannot be construed as showing a causal relationship between heptachlor epoxide and arteriosclerosis because there are no data on the background levels of pesticides in this population, and no adjustments for other risk factors for arteriosclerosis were made.

No studies were located regarding cardiovascular effects in animals after inhalation exposure to heptachlor or heptachlor epoxide.

Hematological Effects. Blood dyscrasias, including production defects and thrombocytopenic purpura, were described in a case report of 25 individuals exposed for an unspecified duration to heptachlor and chlordane following home application for termite treatment (Epstein and Ozonoff 1987). The primary route of exposure was probably inhalation. This study is limited by lack of specific exposure information and concomitant exposure to other pesticides. A case-control study of 60 men who died from aplastic anemia and 120 controls showed no dose-dependent causal relationship between pesticide exposure and aplastic anemia (Wang and Grufferman 1981). The cases were all males who died of aplastic anemia between the ages of 15 and 65 years in the state of North Carolina. The controls selected were men on the mortality list who met the criteria of having died in the same year of causes other than aplastic anemia and of being of the same race and age range at death as the case group. The occupations of all but 4 of the 180 cases and controls were obtained from the death certificates. There were no significant associations between aplastic anemia and occupation, which included exterminators, gardeners, and agricultural workers. However, the pesticide usage was estimated by domestic disappearance, and not direct measurement. Domestic disappearance is calculated by subtracting exports and net changes in inventories from total annual production. Three cases of aplastic anemia associated with exposure to chlordane, which can contain heptachlor, were reported by Infante et al. (1978). The cases were three males aged 15, 28, and 68 years. These exposures were not quantitated and were assumed to be some combination of inhalation

and dermal exposure. There could also have been exposures to other chemicals. Leukemia has also been associated with exposure to heptachlor in case reports. See Section 2.2.1.8.

No studies were located regarding hematological effects in animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.5 Developmental Effects

Placental transfer of heptachlor epoxide was reported by Polishuk et al. (1977a). Heptachlor epoxide concentration in extracted lipids of fetal plasma (0.9959 ppm) exceeded that of the maternal blood sample (0.2798 ppm) or of the uterine muscle. These data indicate that the uterus and placenta do not provide an effective barrier to the fetus for these compounds. Heptachlor epoxide has also been identified in breast milk, thus providing an additional route of exposure for infants. This compound has also been detected in stillborn infant brain, adrenal, lung, heart, liver, kidney, spleen, and adipose tissues (Curley et al. 1969). Other than determining that the women had no known direct exposure to pesticides, the authors did not attempt to quantitate maternal heptachlor exposure levels. These studies are limited by the lack of data concerning route, duration, extent of exposure, and number of cases examined. No gross malformations were described in any of the stillborn infants. Although a developing organism could potentially be exposed to heptachlor transplacentally or during lactation, the existing data are inadequate to establish a relationship between exposure to heptachlor or heptachlor epoxide and human developmental toxicity.

No studies were located regarding developmental effects in animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.6 Reproductive Effects

Significantly higher levels of heptachlor epoxide were detected in the sera of a group of women identified through hospital records with premature delivery than in the sera of a control group with normal delivery (Wassermann et al. 1982). However, sera levels of 8 of the 10 organochlorine pesticides for which analytical data were obtained were all significantly higher in the premature delivery group. In addition, route, duration, and level of exposure information was not reported. Heptachlor epoxide has been reported in stillborn infant brain, adrenal, lung, heart, liver, kidney, spleen, and adipose tissue, indicating transplacental transfer of heptachlor or heptachlor epoxide (Curley et al. 1969). These studies also reported the presence of polychlorinated biphenyls, lindane, and dieldrin in the samples. Lack of control for confounding factors such as smoking and concomitant exposure to other pesticides and lack of completeness of report data make it difficult to assess the causal relationship between adverse reproductive outcome in humans and inhalation exposure to heptachlor and heptachlor epoxide.

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No studies were located regarding reproductive effects in animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide.

Genotoxicity studies are discussed in Section 2.4.

2..2.1.8 Cancer

A series of case reports described five cases of neuroblastoma and three cases of acute leukemia associated with chlordane exposure (Infante et al. 1978). In two of the cases of neuroblastoma, the exposure to chlordane occurred during prenatal development; otherwise, the exposures .are assumed to be via inhalation in combination with dermal contact that occurred at the ages of 1 year and 11 months, 2 years and 5 months, and 3 years and 8 months. The developmental period during which the exposures occurred for the cases of leukemia was not specified. Dosages and durations of exposure were also not specified. The authors concluded that there is an association between chlordane exposure and neuroblastoma and between chlordane exposure and leukemia but did not quantify the exposure frequency. However, the association of these malignancies with heptachlor exposure cannot be confirmed from these data because the exposures were to chlordane and were not quantified. In another study, leukemia was associated with exposure to chlordane and heptachlor following home termiticide use. However, this exposure cannot be confirmed to be causal because the study was limited by concomitant exposure to other chemicals, lacked quantitative exposure data, and failed to adjust for other potential causal factors such as genetic disposition or immunologic disorders (Epstein and Ozonoff 1987).

In a large occupational cohort mortality study, 16,124 workers engaged in the manufacture of chlordane and heptachlor were followed for cause of death (Wang and MacMahon 1979a). The results of the original study found no significant increase in death from any type of cancer. The SMR for bladder cancer was of borderline significance, but no information on cigarette smoking was obtained from the participants. The follow-up of this cohort of pesticide applicators identified an increase in lung cancer, but the SMR for deaths from lung cancer for the manufacturing group with the highest chance of exposure to heptachlor was only 97, indicating no increase for lung cancer deaths in this group and suggesting that chlordane and heptachlor were not responsible for the lung cancer increase. It is possible that the sample size was not sufficient for detecting the effect by the statistical methods employed in the study. In addition, the excess in lung cancers occurred in persons employed for less than 5 years, which suggests that factors other than prolonged exposure to heptachlor were responsible (MacMahon et al. 1988).

A retrospective mortality study was conducted on 1,403 white male workers engaged in chlordane, heptachlor, and endrin manufacture between 1946 and 1976 (Wang and MacMahon 1979b). All subjects were employed for at least 3 months. A slight excess of lung cancer was seen in this cohort compared to the general U.S. population, but the increase was not statistically significant. The lack of complete occupational histories made it impossible to examine the relationship between exposure intensity and mortality. The study is also limited by lack of quantitative exposure information, concomitant exposure to other chemicals, and lack of control measures for confounding factors.

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An occupational mortality study conducted on a cohort of workers employed for at least 3 months between 1952 and 1979 at a Velsicol plant in Memphis, Tennessee, revealed no pattern of disease or medical condition that indicated that persons employed in the manufacture of chlordane and other chlorinated hydrocarbon pesticides were at greater risk of adverse outcome than the general population (Shindell and Associates 1981). In general, the workers at the plant demonstrated the healthy worker effect as evidenced by a lower incidence of cancer and other health effects compared to the control population. This study had several deficiencies. The study design did not include examination of employees by designated physicians to establish manifestations such as respiratory difficulties, anxiety, restlessness, headache, etc. The blood and urine were not analyzed to verify the presence of pesticide, and serum was not obtained to determine serum glutamic-pyruvic transaminase (SGPT) or serum glutamic-oxaloacetic transaminase (SGOT) levels. Pregnancy status and race of women employees were not determined. Many of the workers were exposed to other chemicals in addition to heptachlor. The level of exposure to heptachlor was not documented. This study achieved 92.8% complete follow-up, suggesting that these findings do in fact represent the experience of the cohort as a whole. However, it would be desirable to conduct a follow-up study on the same population.

No studies were located regarding cancer in animals after inhalation exposure to heptachlor or heptachlor epoxide.

2..2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to heptachlor or heptachlor epoxide. However, since heptachlor is a major component of the insecticide chlordane, chlordane poisoning can be considered when evaluating heptachlor toxicity data. In the case study of a woman who ingested 6 g of chlordane with suicidal intent and died 9.5 days following ingestion, no information was presented on the composition of the chlordane. Therefore, the amount of heptachlor exposure is unknown, and the effect of other components of chlordane cannot be ruled out (Derbes et al. 1955).

Acute oral LD_{50}s for heptachlor in rodents (rats, mice, hamsters, and guinea pigs) and rabbits range from 40 to 162 mg/kg (purity ranging from unspecified to 99.9%) (Ben-Dyke et al. 1970; Eisler 1968; Gaines 1969; Gak et al. 1976; Lehman 1951; Podowski et al: 1979; Sun 1972). Acute oral LDS_{50}s for heptachlor epoxide in rodents (rats and mice) and rabbits range from 39 to 144 mg/kg (Eisler 1968; Podowski et al. 1979). The studies provide little information on procedural details such as dosing, number of doses, and detailed results. AI1 studies except Gak et al. (1976) and Sun (1972) used gavage dosing. The number of animals tested was either small or not reported.

Two calves receiving 2.5 or 5 mg/kg/day of heptachlor formulation (25% heptachlor) for 15 or 6 days, respectively, died after the last doses were administered (Buck et al. 1959). In contrast, among six calves given single doses of heptachlor epoxide formulation (25% heptachlor epoxide), two received 25 mg/kg, and three received 15, 10, or 5 mg/kg/day. All died within 3 hours to 3 days. These results indicate that heptachlor epoxide is more toxic to young calves than technical-grade heptachlor.

Heptachlor can be converted to its photoisomer, photoheptachlor, in the presence of sunlight or ultraviolet light. This photolysis can take place on plant leaves. Despite the use of a small number of test animals, photoheptachlor was found to be more toxic to rats than heptachlor or heptachlor epoxide. The LD_{50} for photoheptachlor was 3.8 mg/kg (Podowski et al. 1979).

Groups of eight male minks were fed diets containing 0, 1.79, 3.11, 5.67, or 6.19 mg/kg/day heptachlor for 28 days. Three minks receiving the highest dose died, two of them in the post-exposure observation period (Aulerich et al. 1990). Thus, intermediate exposure to heptachlor was highly toxic to minks.

Groups of 10 adult Osborne-Mendel rats (5/sex) and 10 adult B6C3F_1 mice (5/sex) were fed technical-grade heptachlor in food (73% heptachlor, 22% chlordane, 5% nonachlor) for 6 weeks, followed by a 2-week period of observation. Dietary doses were 1, 2, 4, 8, and 16 mg/kg/day (NCI 1977) for the rats and 2.6, 5.2, and 10.4 mg/kg/day for the mice. Two of five male rats died at the highest dose; no deaths were reported at 8 mg/kg/day or less. The LOAEL for male rats was 16 mg/kg/day, and the NOAEL was 8 mg/kg/day. All of the female rats died at the 16-mg/kg/day level, and four of five died at the 8-mg/kg/day level. No deaths were reported at 4 mg/kg/day or less. All male mice died at the l0.4-mg/kg/day level, the highest dietary dose tested in mice. No deaths were reported at levels of 5.2 mg/kg/day or lower. Two of five female mice died at the highest dose; no deaths were reported at the lower doses.

Groups of 50 male and 50 female B6C3F, mice were fed diets containing technical-grade heptachlor (73% heptachlor, 22% chlordane, 5% nonachlor) for up to 80 weeks at time-weighted average (TWA) doses of 0.79 or 1.8 mg/kg/day for males and 1.1 or 2.3 mg/kg/day for females. Following treatment, the animals were observed for 10 weeks. There were no significant differences in survival between the control and treated males. In females, there was a dose-related increase in mortality, due mainly to the effect of the high dose (NCI 1977). The increased mortality in females could be due to greater susceptibility in females or to the larger dose received by females. Chronic exposure of 50 male and 50 female Osborne-Mendel rats fed the same compound for 80 weeks at TWA doses of 1.94 or 3.9 mg/kg/day for males and 1.28 or 2.56 mg/kg/day for females resulted in a 20% decrease in survival in high-dose females (NCI 1977).

A high incidence (55-60%) of mortality was reported in neonatal rats following dietary exposure of parental rats for 18 months (Mestitzova 1967).

All reliable LD_{50} values and ail reliable LOAEL values for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.2 Systemic Effects

No studies were located regarding respiratory effects in humans or animals ‘after oral exposure to heptachlor or heptachlor epoxide.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species ‘and duration category are recorded in Table 2-l and plotted in Figure 2-1.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to heptachlor or heptachlor epoxide. In an intermediate-duration study, increased heart-to-body-weight ratio was reported in rats following dietary exposure to 0.5 mg/kg/day heptachlor, 5 days/week, for 4 weeks (Enan et al. 1982).

Gastrointestinal Effects. Nausea and vomiting were reported in humans following accidental ingestion of chlordane (Dadey and Kammer 1953; Derbes et al. 1955). These symptoms developed within 1.5-2.5 hours after a one-time ingestion of chlordane. Histopathologic ex,amination showed that the stomach and intestimal walls were slightly hyperemic in rats exposed to 5 mg/kg/day of heptachlor for 28 days (Pelikan 1971).

TABLE 2-1. Levels of Significant Exposure to Heptachlor and Heptachlor Epoxide - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
ACUTE EXPOSURE									
Death									
1	Rat	(GO)	1 d 1x/d				71 (LD50) 60 (LD50) 3.8 (LD50)	Podowski et al. 1979	H HE PH
2	Rat	(GO)	1 d 1x/d				100 (LD50 male) 162 (LD50 female)	Gaines 1969	H
Systemic									
3	Rat	(GO)	1 d 1x/d	Hepatic		60 (increased serum GPT and ALD, increased liver GPT and ALD at 2 hours, decreased liver GPT and ALD at 72 hours, vacuolated cells, pyknotic nuclei)		Krampl 1971	H
Reproductive									
4	Mouse	(G)	1 d 1x/d		15			Arnold et al. 1977	H/HE
5	Mouse	(G)	5 d 1x/d		8			Epstein et al. 1972	HE
6	Mouse	(G)	5 d 1x/d		10			Epstein et al. 1972	H
INTERMEDIATE EXPOSURE									
Death									
7	Rat	(F)	6 wk ad lib				16 (2/5 M) 8 (4/5 F)	NCI 1977	H

TABLE 2-1 (Continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
8	Mouse	(F)	6 wk ad lib				10.4 (5/5 M) 10.4 (2/5 F)	NCI 1977	H
9	Mink	(F)	28 d ad lib				6.19 (3/8 M)	Aulerich et al. 1990	H
Systemic									
10	Rat	(F)	28 d ad lib	Hepatic		5 (steatosis, 19% increase in liver weight)		Pelikan 1971	H
				Gastro		5 (slight hyperemia of stomach and intestinal walls)			
11	Rat	(F)	4 wk 5d/wk 1x/d	Hemato		0.5 (37% increase in WBC after 7 days, 70% increase in WBC after 28 days)		Enan et al. 1982	H
				Hepatic		0.5 (increased levels of bilirubin, glucose, and acid phosphatase at 7 days, decreased glycogen at 7 days, increased cholesterol and AP at 28 days, increased liver weight)			
				Renal		0.5 (87% increase in blood urea at 7 days)			
12	Rat	(GO)	28 d 1x/d	Hepatic		7 (mononuclear necrosis, GPT and ALD decreased in liver and increased in serum, normal ALD levels by 28 days)		Krampl 1971	H

TABLE 2-1 (Continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
13	Mouse	(W)	180 d ad lib	Hepatic		5.7 (increased levels of SGPT, and liver lipid peroxide, increased liver to body weight ratio)		Izushi and Ogata 1990	H
				Musc/skel		5.7 (increased serum creatinine phosphokinase)			
14	Mouse	(F)	10 wk 7d/wk 4x/d	Hepatic			6.5 (hepatitis, necrosis, granuloma, congestion)	Akay and Alp 1981	H
				Renal			26 (granuloma)		
15	Mouse	(W)	26 d	Other			80 (adrenal fibrosis, cortical cell granulation, lipid accumulation)	Akay et al. 1982	H
16	Mouse	(GO)	92 d 2x/wk	Hepatic		10 (increased SGPT, serum AP, liver triglycerides, and liver to body weight ratio)		Izushi and Ogata 1990	H
17	Pig	(F)	78 d 1x/d	Hepatic		2 (decreased glycogen)		Halacka et al. 1974	H
				Other	2	5 (16% decrease in body weight gain)			
18	Pig	(F)	78 d 1x/d	Hepatic		2 (increased agranular endoplasmic reticulum in liver cells, decreased glycogen content)		Dvorak and Halacka 1975	H

TABLE 2-1 (Continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
19	Mink	(F)	28 d ad lib	Hepatic	5.67	6.19 (fatty infiltration of the liver)		Aulerich et al. 1990	H
				Renal	5.67	6.19 (granulation, kidney discoloration, decreased kidney to body weight ratio)			
				Other		5.67 (22% decrease in body weight)			
Immunological									
20	Rat	(F)	28 d ad lib			5 (enlarged, congested hyperemic spleen)		Pelikan 1971	H
21	Mouse	(F)	10 wk 7d/wk 4x/d				26 (splenic fibrosis, increased spleen erythrocytes and eosinophilic leukocytes)	Akay and Alp 1981	H
22	Mink	(F)	28 d ad lib		3.11	6.19 (49% decrease in spleen/brain weight)		Aulerich et al. 1990	H
Neurological									
23	Mouse	(F)	10 wk 7d/wk 4x/d		6.5		13 (ataxia, tremors, self-mutilation, in females)	Akay and Alp 1981	H
24	Mink	(F)	28 d ad lib		5.67	6.19 (hyperexcitability, incoordination, paralysis in hind quarters)		Aulerich et al. 1990	H
Developmental									
25	Rat	(F)	60 d				0.25 (16% embryo survival in F1 generation)	Green 1970	H

TABLE 2-1 (Continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
Reproductive									
26	Rat	(F)	60 d				0.25 (F1 generation: 30% decrease in fertility, increased resorption, F2 generation: 100% infertility)	Green 1970	H
27	Mouse	(F)	10 wk 7d/wk 4x/d				6.5 (100% infertility)	Akay and Alp 1981	H
CHRONIC EXPOSURE									
Death									
28	Rat	(F)	80 wk ad lib				2.56 (20% decrease in survival of females)	NCI 1977	H
29	Mouse	(F)	80 wk ad lib				2.3 (18% decrease in survival of females)	NCI 1977	H
Systemic									
30	Rat	(F)	18 mo 1x/d	Ocular			6 (lens cataracts)	Mestitzova 1967	H
Developmental									
31	Rat	(F)	18 mo 1x/d				6 (55-62% neonatal, death)	Mestitzova 1967	H
Reproductive									
32	Rat	(F)	80 wk ad lib			1.28 (vaginal bleeding)		NCI 1977	H

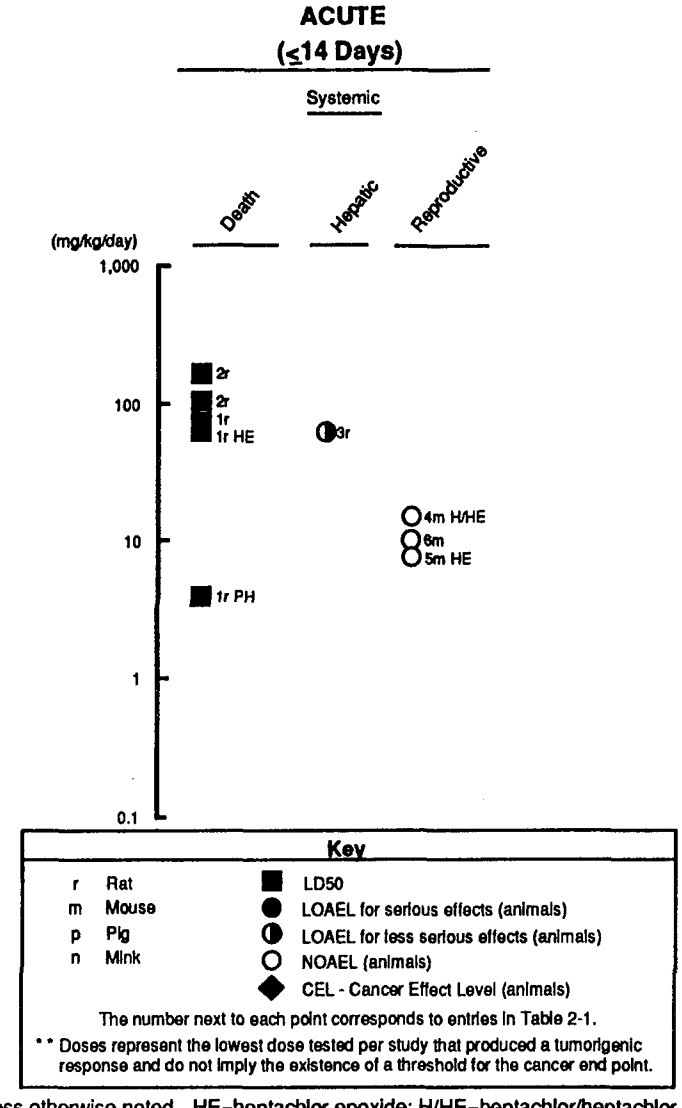
TABLE 2-1 (Continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Compound
33	Rat	(F)	18 mo 1x/d				6 (23% decrease in mean litter size, 57% mortality at one month)	Mestitzova 1967	H
Cancer									
34	Mouse	(F)	80 wk ad lib				1.8 (hepatocellular carcinoma in males) 2.3 (hepatocellular carcinoma in females)	NCI 1977	H

[a]The number corresponds to entries in Figure 2-1.

ad lib = ad libitum; ALD = aldolase; AP = alkaline phosphatase; d = day(s); F = female(s); (F) = feed; F1 = first filial generation; F2 = second filial generation; (G) = unspecified gavage; Gastro = gastrointestinal; (GO) = oil gavage; GPT = glutamate pyruvic transaminase; H = heptachlor; HE = heptachlor epoxide; H/HE = mixture of heptachlor and heptachlor epoxide; Hemato = hematological; (LD50) = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; PH = photoheptachlor; SGPT = alanine aminotransferase; (W) = water; WBC = white blood cell(s); wk = week(s); x = time(s)

FIGURE 2-1. Levels of Significant Exposure to Heptachlor and Heptachlor Epoxide - Oral *



* All exposures were to heptachlor unless otherwise noted. HE=heptachlor epoxide; H/HE=heptachlor/heptachlor epoxide mixture; PH=photoheptachlor

FIGURE 2-1 (Continued)

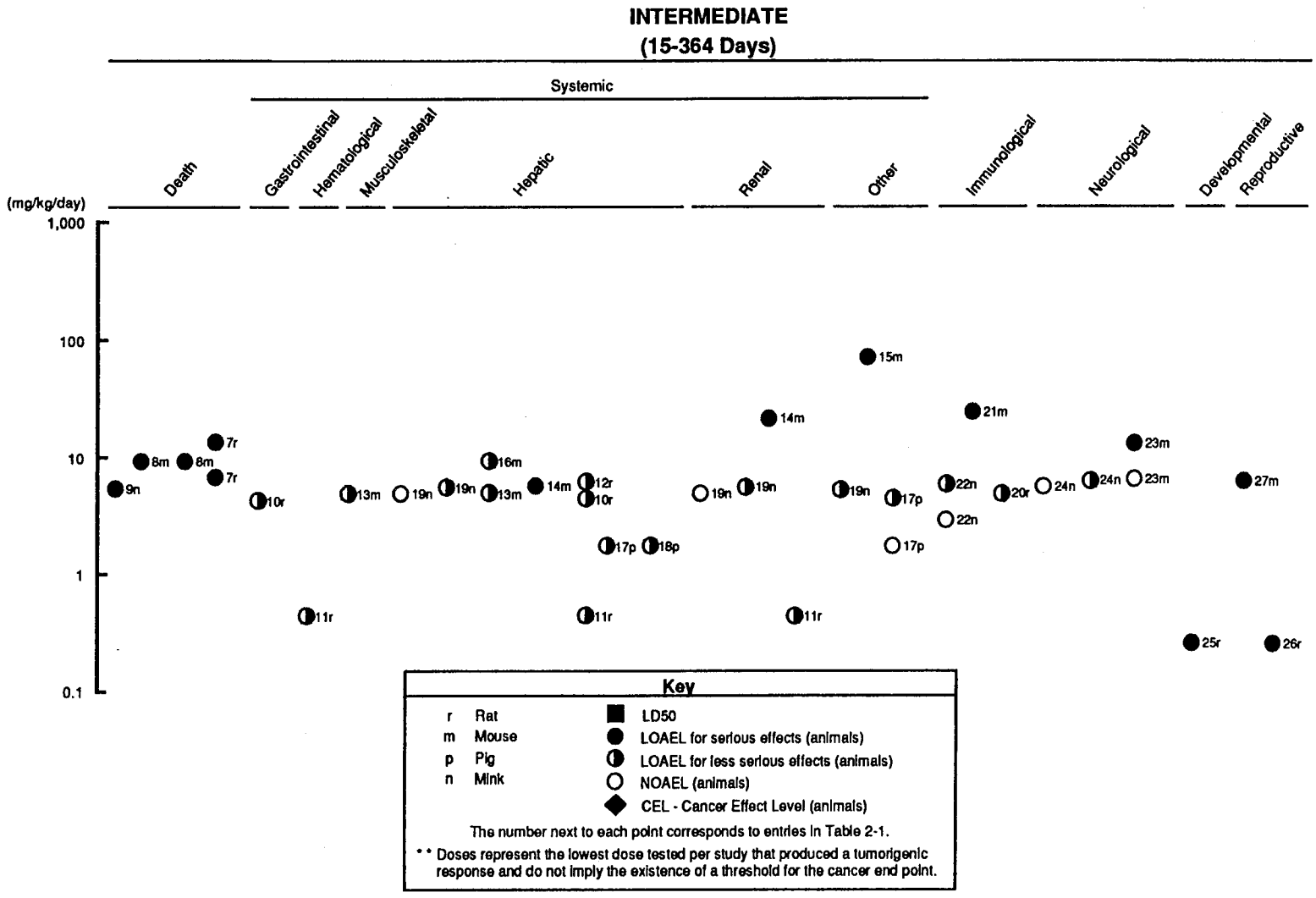
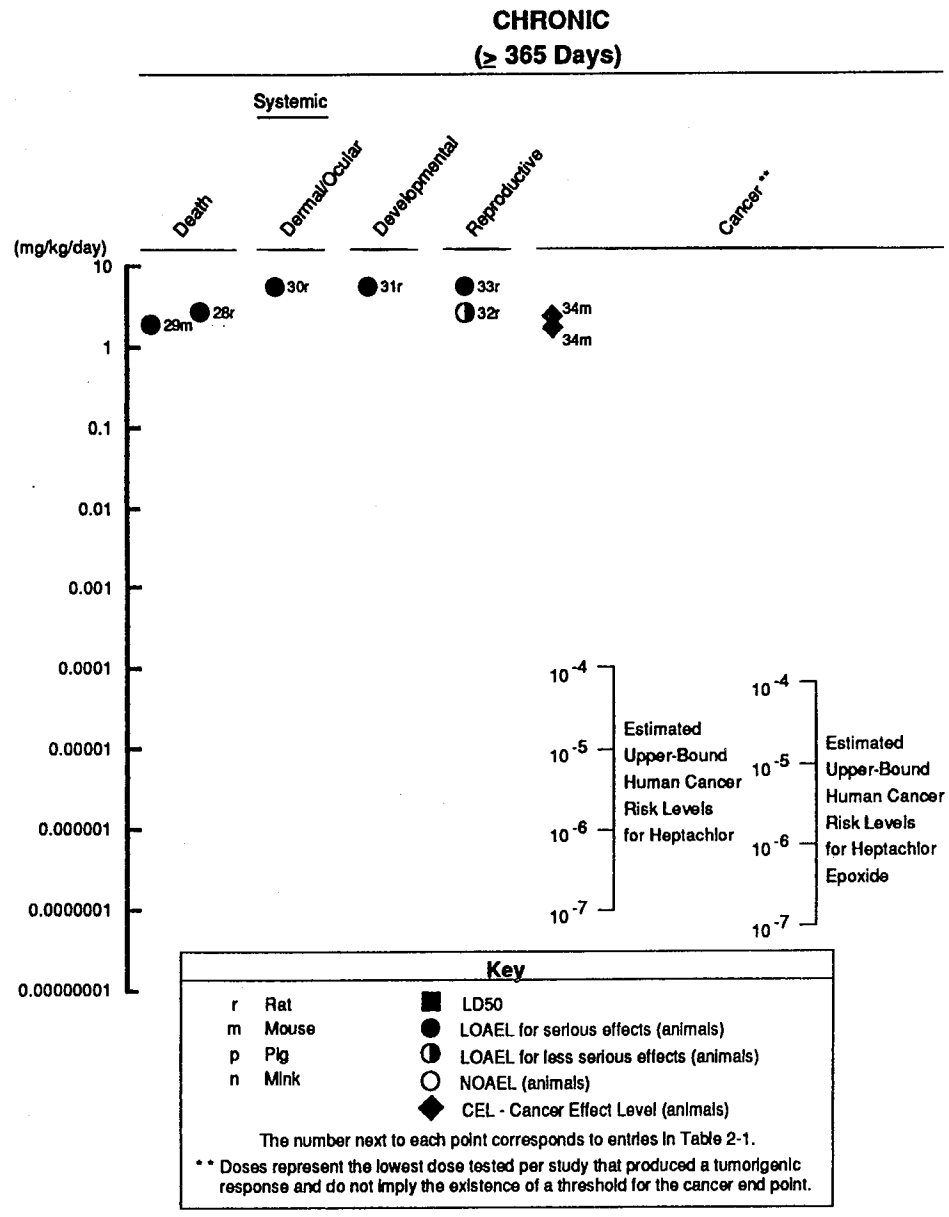


FIGURE 2-1 (Continued)



2. HEALTH EFFECTS

Calves fed multiple doses of heptachlor (2.5, 5, or 10 mg/kg/day for 16, 6, and 3 days, respectively) or heptachlor epoxide (2.5 and 3.5, or 15 mg/kg/day for 3 or 5 days, respectively) had hyperemic or hemorrhagic gastrointestinal tracts (Buck et al. 1959).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to heptachlor or heptachlor epoxide. See Section 2.2.1.2 for information on hematological effects from exposures thought to be by the inhalation route.

Rats that received 0.5 mg/kg/day of heptachlor (96% purity) in the diet in an intermediate-duration study (5 days/week for 4 weeks) showed a statistically significant increase in total white blood count and bilirubin at 1, 7, and 28 days post-exposure (Enan et al. 1982). This study is limited by the use of insufficient dose levels to establish a dose response.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to heptachlor or heptachlor epoxide. Calves given multiple doses of heptachlor (2.5, 5, or 10 mg/kg/day for 15, 6, and 3 days, respectively) and heptachlor (2.5, and 3.5, or 15 mg/kg/day for 3 and 5 days, respectively) exhibited muscle spasms as secondary effects to central nervous stimulation (Buck et al. 1959). In another study, an increase in serum creatinine phosphokinase was observed in mice fed 5.7 mg/kg heptachlor for 180 days (Izushi and Ogata 1990). This suggests that muscle damage may have occurred, but supporting histopathology was not presented by the authors.

Hepatic Effects. In a study of 45 individuals exposed for an unspecified period of time to contaminated raw milk products from cattle fed heptachlor-contaminated feed, 23-31% were found to have significantly elevated serum levels of heptachlor metabolites. Results of liver function tests and assays for hepatic microsomal enzyme induction did not differ from those of the local comparison cohort (Stehr-Green et al. 1986). In a follow-up study of the same families approximately 18 months later, heptachlor epoxide was found in the blood of 7 out of 39 subjects who drank raw milk contaminated with heptachlor at concentrations as high as 89.2 ppm and in the blood of 3 out of 79 controls. The exposed group had significantly higher mean serum levels of heptachlor epoxide (0.84 ppb) compared to the control group (0.50 ppb). However, no evidence of related acute or subacute hepatic effects such as hepatomegaly was found in the exposed subjects, regardless of their serum residue concentrations (Stehr-Green et al. 1988).

Oral exposures in rats and mice have been shown to increase hepatic micxosomal enzymes (Den Tonkelaar and Van Esch 1974; Krampl 1971) and to alter hepatic carbohydrate metabolism (Enan et al. 1982; Kacew and Singhal 1973). At week 1, the blood glucose levels increased by 48% while liver glycogen decreased. AlI measured liver gluconeogenic enzymes were increased over control levels by 41% (Kacew and Singhal 1973). This study, however, used only one dose that was above the LD_{50}, and only four animals per group were used. In addition, the dose is above the solubility limit for heptachlor, so there is some question as to the actual dose administered Single oral exposure of heptachlor (60 mg/kg) to female rats increased serum and liver GPT and ALD at two hours and decreased liver GPT and ALD at 72 hours; histology revealed vacuolated cells with pyknotic nuclei. Histologic examination of liver tissue from female rats given 7 or 12 mg/kg/day heptachlor (98% purity) for 28 days revealed slight morphologic changes that increased with increasing dose. The authors concluded that there could be a correlation between cellular leakage and necrosis and serum enzyme levels (Krampl 1971). Increased liver weight and an increase in hepatic lipid content occurred in rats that received 5 mg/kg/day heptachlor for 14 or 28 days (Pelikan 1971). No clinical signs were observed in any of the groups. This study used only one dose level and a control and did not report statistical significance. Mice that received 6.5, 13, or 26 mg/kg/day heptachlor showed toxic hepatitis with liver granuloma, hepatic cellular degeneration,

necrosis, fibrosis, and congestion in all treatment groups (Akay and Alp 1981). No statistical Lanalyses were presented and microsomal enzymes were not measured.

Three pigs receiving 2 or 5 mg/kg/day heptachlor showed a depletion of liver glycogen and increased agranular endoplasmic reticulum beginning at 78 and 27 days of exposure, respectively. Pigs receiving 5 mg/kg/day also showed an increase in lysosomes (Dvorak and Halacka 1975; Halacka et al. 1974). Activity of the 5th fraction of liver lactate dehydrogemase increased at the highest dose, as did swelling of the liver (without a change in liver weight) and slight steatosis of the hepatocytes (Halacka et al. 1974). Fatty infiltration of liver was observed in minks fed 6.19 mg/kg/day heptachlor for 28 days (Aulerich et al. 1990).

Oral exposure of mice to heptachlor for 92 days (10 mg/kg/day) or 180 days (5.7 mg/kg/day) increased SGPT and decreased phospholipids and total serum cholesterol (Izushi and Ogata 1990). Triglyceride content wIas increased at 92 days only. Evidence of liver damage was seen as a significant increase in SGPT. An increase in the liver-to-body-weight ratio was also observed.

Physiological responses following chronic dietary administration of heptachlor epoxide were investigated in five groups of beagle dogs (2 males and 3 females per group) fed diets containing 0, 0.013, 0.062, 0.13, or 0.19 mg/kg/day heptachlor epoxide continuously for 60 weeks (University of Cincinnati 1958). The body weight gain of male dogs decreased with the increasing concentration: this effect was margimally significant. There was a statisticdly significant, dose-dependent increase in termimal liver weights in both sexes of dogs. However, this increase wras not accompanied by histological changes and, therefore, could have been an adaptive response to treatment-related toxicity. No treatment-related clinical signs were noted. Moreover, the animals suffered from pneumonia which suggests poor animal husbandry. The other study limitations included an insufficient number of animals for meaningful statistical analysis, improper diet preparation, lack of analytical chemistry data, short experimental duration, and individual variations among animals reflecting genetic variability of the dog colony stock. Thus, chronic exposure to low concentrations of heptachlor epoxide produced minimal physiological changes in beagle dogs.

Renal Effects. Urimary output was severely reduced and uremia was present in a woman 24 hours after intentional ingestion of about 6 g of chlordane. After 9.5 days, she died; autopsy revealed nephrosis of the kidneys (Derbes et al. 1955).

Heptachlor was shown to alter renal carbohydrate metabolism in male Wistar rats that received a single dose of 200 mg/kg heptachlor. All measured gluconeogenic enzymes in the kidney cortex were significantly increased compared to controls. Heptachlor also elevated cyclic adenosine monophosphate (AMP) levels in the kidney cortex (Kacew and Singhd 1973). Granulomas were observed in the kidneys of mice that received 26 mg heptachlor/day in an intermediate-duration study (Akay and Alp 1981). Granuloma is a general term used to describe modular inflammation lesions that frequently contain proliferated macrophages. The inflammation characteristics as well as the increase in macrophages suggest some immune involvement, which is supported by the observation of splenic fibrosis.

Granulation and discoloration of kidneys and a decrease in kidney-to-brain-weight ratio was reported in minks fed 6.19 mg/kg/day of heptachlor daily for 28 days (Aulerich et al. 1990). Rats receiving 0.5 mg/kg/day of heptachlor in the diet in an intermediate-duration study showed a statistically significant increase in blood urea (Enan et al. 1982). Increased blood urea may indicate renal inefficiency in metabolism and clearance of protein by-products. This study is limited in that histologic examination was not included in the study design and insufficient dose levels were utilized to establish a dose response.

2. HEALTH EFFECTS

Dermal/Ocular Effects. No studies were located regarding dermal/ocular effects in humans after oral exposure to heptachlor or heptachlor epoxide.

Of 50 adult rats used in a reproductive/developmental study, 22% of those that received 6 mg/kg/day heptachlor in the diet developed lens cataracts 4.5-9.5 months following exposure. In addition, 6-8% of the F_1, offspring and 6% of the F_2 offspring of these rats also developed cataracts 19-21 days after birth (Mestitzova 1967). The author of this study eliminated the possibility of a vitamin B deficiency or a recessive genetic trait as the cause of the cataracts. She could not rule out the possibility of altered vitamin B metabolism caused by heptachlor.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after oral exposure to heptachlor or heptachlor epoxide. A reduction in body weight was reported in minks fed heptachlor in the diet for 28 days (Aulerich et al. 1990). The observed effects were proportional to the concentration of heptachlor in the diet. Three pigs receiving 5 mg/kg/day of heptachlor for 78 days had a 16% decrease in body weight gain compared to controls (Halacka et al. 1974). Female mice receiving 80 mg/kg/day heptachlor (89% purity) showed increased incidences of cortical atrophy and slight hypertrophy in the zona glomerulosa of the adrenal gland compared to controls. Heavy lipid accumulation and granulation were observed in cortical cells on day 26 of exposure. Congestion, cell degeneration, and fibrosis in the adrenal cortex were reported at the end of the study in treated mice only; lack of these effects in controls suggest that they were not stress related (Akay et al. 1982). The interpretation of these findings is limited because the l00-ppm concentration reportedly used exceeds the solubility of heptachlor in water (0.05 ppm) (EPA 1987a). This implies that either the dose was reported incorrectly or that the heptachlor was present in suspension, calling into question the uniformity of dosing.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans after oral exposure to heptachlor or heptachlor epoxide.

No animal studies were located that specifically investigated the effects on the immune system of oral exposure to heptachlor. However, systemic findings in three studies included some reference to changes that may reflect an effect on the immune system. Wistar rats fed 5 mg/kg/day heptachlor for 28 days developed enlarged, congested, and hyperemic spleens (Pelikan 1971). Female rats fed 0.5 mg/kg/day heptachlor for 4 weeks showed a significant increase in white blood cell count and an increased spleen-to-body-weight ratio (Enan et al. 1982). Mice fed 26 mg/kg/day heptachlor in food for 10 weeks showed kidney and liver granuloma, splenic fibrosis, and an increase in the number of erythrocytes and eosinophilic leukocytes in the spleen (Akay and Alp 1981). A decreased spleen-to-brain-weight ratio was reported in minks receiving 6.19 mg/kg/day heptachlor in the diet for 28 days (Aulerich et al. 1990).

The highest LOAEL values for immunological effects in each species following intermediate exposure are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.4 Neurological Effects

A case report of oral exposure to technical-grade chlordane reported neurological effects including irritability, salivation, dizziness, muscle tremors, and convulsions (Dadey and Kammer 1953). However, exposure measurements were not provided in the report, and technical-grade chlordane contains varying amounts of heptachlor. The effects cannot be said to have resulted from exposure to heptachlor only.

Tremors and convulsions were reported to occur in rats given 90 mg/kg heptachlor (oral LD_{50}) in a single dose. Neurotoxic signs appeared 30-60 minutes after dosing and lasted 2 days (Lehman 1951). This study was limited because procedural details were omitted, compound purity was not reported, and the number of animals tested was not reported. Hyperexcitability and incoordination were reported in minks fed 6.19 mg/kg/day heptachlor for 28 days; one had paralysis of the hind legs (Aulerich et al. 1990). Mice that received 13 mg/kg/day heptachlor for 10 weeks had difficulty in walking and standing and lost the righting reflex. Whole-body tremors and self-mutilation also occurred (Akay and Alp 1981).

Statistically significant changes in electroencephalogram (EEG) patterns were reported in female adult Wistar rats administered heptachlor in the diet at levels of 1 and 5 mg/kg/day for three generations (Formanek et al. 1976). Interpretation of these findings is difficult because details of the dosing, the procedures used, and conditions of the rats were not described.

Young calves fed multiple doses of heptachlor (2.5, 5, or 10 mg/kg/day for 15, 6, and 3 days, respectively) or heptachlor epoxide (2.5 and 3.5, or 15 mg/kg/day for 3 and 5 days, respectively) had muscle spasms in the head and neck region, convulsive seizures, elevated body temperatures, and engorged brain blood vessels (Buck et al. 1959).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species following intermediate exposure are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.5 Developmental Effects

The only study located regarding developmental effects reported no adverse effects on human fetal development following transplacental exposure to heptachlor based on birth certificate information and hospital discharge data (Le Marchand et al. 1986). The study was conducted on women of child-bearing age from Oahu, Hawaii, who ingested milk containing heptachlor for 27-29 months. The study did not provide data on the heptachlor level in the milk of nonexposed women. Therefore, the data are inadequate to establish a relationship between exposure to heptachlor and human developmental toxicity. Milk fat levels of heptachlor measured in Hawaii during this time ranged from 0.12 to 5.00 ppm (EPA's "worst case" estimates on record range from 0.10 to 1.20 ppm). No increase in fetal or neonatal deaths or incidence of low birth weight infants were found in this study cohort. Of the 23 categories of major congenital malformations evaluated, 22 were found to be decreased in the study population when compared with comparison cohorts from the other Hawaiian islands and from the U.S. general population for the same time period. One type of malformation (anomalies of the abdominal wall) was found to be slightly increased in the study cohort during the period of known exposure compared with the control cohorts. However, the baseline data for this type of malformation were not available prior to study initiation, and birth defects may be underreported. It was, therefore, not possible to document the temporal change in the incidence of this type of malformation. Since women who might not have consumed the contaminated milk were included in the study group, positive findings may have been diluted as a result of misclassification bias.

Cataracts and decreased postnatal survival were reported in the progeny of rats fed diets containing heptachlor. However, the data were insufficient to further evaluate these studies. Because cataracts also developed in the adult rats post-exposure, there is reason to question whether cataracts actually are a developmental effect. These studies are discussed below.

Transplacental exposure to heptachlor (98% purity) also significantly shortened the life-span of sucklings with the death rate being highest in the first 24-48 hours (Mestitzova 1967). Cataracts were noted in the progeny 19-21 days after eye opening. This study had several deficiencies including lack of details regarding the strain and number of rats, dosing methodology, duration of treatment period, and statistical analysis. Use of a single dose level precludes assessment of dose response.

Four male and 15 female Sprague-Dawley rats were fed diets containing 0.25 mg/kg/day heptachlor (purity not reported) for 60 days prior to mating and treatment continued during gestation of the females (Green 1970). Reduced fertility and increased resorptions were seen in the treated group, but statistical significance was not reported. The number of abnormal embryos was not significantly different. Postnatal survival in the F, progeny was reduced. Only 19 out of 122 offspring of treated rats survived 21 days postpartum compared to 179 out of 288 offspring of controls. The LOAEL for decreased embryo survival was 0.25 mg/kg/day. The study was conducted using only one dose level, and therefore, a NOAEL was not established.

The reliable LOAEL values for developmental effects in rats following intermediate and chronic exposure are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.2.6 Reproductive Effects

No adverse effects on reproduction (no decrease in fertility, no increase in fetal or neonatal deaths) were reported by Le Marchand et al. (1986) among women of child-bearing age following ingestion of heptachlor-containing milk in excess of 0.1 ppm for 27-29 months.

In a dominant lethal assay, eight male Charles River CD-l mice received single oral doses of 7.5 or 15 mg/kg/day of a heptachlor:heptachlor epoxide mixture (25%:75%) and were bred with three untreated females each week for 6 weeks (Arnold et al. 1977). No adverse effect on the reproductive capacity of male mice was noted; therefore, the NOAEL was 15 mg/kg. A LOAEL was not established. Both heptachlor and heptachlor epoxide were also tested separately in another dominant lethal assay in mice. Heptachlor was tested at 5 and 10 mg/kg and heptachlor epoxide at 8 mg/kg/day. Neither agent produced early fetal deaths or preimplantation losses outside the control limits (Epstein et al. 1972).

Male and female Sprague-Dawley rats were fed diets containing 0.25 mg/kg/day heptachlor for 60 days; the females continued receiving the test diet through gestation (Green 1970). Increased numbers of resorptions were seen, although the number of abnormal embryos was not increased. During the second phase of the study, rats receiving 0.25 mg/kg/day for two generations showed a marked decrease in pregnancy rates. In the first generation, only 18 out of 25 heptachlor-treated females (compared to 30 out of 32 controls) became pregnant. In the second generation, none of 12 females receiving heptachlor became pregnant. Treatment seems to be more likely to affect male than female rats; treated females conceived and had normal litters when bred to males fed control food. The absence of normal viable sperm in the vaginal smear of heptachlor-fed females after copulation and the presence of normal spermatogenesis in the testes suggest that sperm are possibly killed (Green 1970). The LOAEL for decreased fertility in females was 0.25 mg/kg/day. A NOAEL was not established.

Male and female mice that were fed 6.5, 13, or 26 mg/kg/day of heptachlor for 10 weeks failed to produce a new generation after the 10 weeks of exposure (Akay and Alp 1981). No microscopic alterations were found in ovaries or testes. The study was limited by lack of details and statistical analysis.

When rats were fed 6 mg/kg/day heptachlor (98% purity) for an unspecified portion of an l8month study, there was a 23% decrease in size of successive generations (Mestitzova 1967). Vaginal bleeding was reported in rats fed 1.28 mg/kg/day heptachlor for 80 weeks (NCI 1977).

In a 2-year chronic rat study, daily dietary exposure of rats (20/sex) to heptachlor at concentrations of 0, 0.38, 0.075, 0.125, 0.175, and 0.25 mg/kg/day resulted in a failure of animals to reproduce (Witherup et al. 1955). Because of the lack of confirmation of copulation plugs, this effect cannot be definitely attributed to heptachlor exposure. The variations in weaning weight were inversely related to the numbers of pups nursed by the mothers. Growth of the offspring during the preweaning period was normal. Treatment-related high mortality was noted among offspring of mothers fed heptachlor at 0.125, 0.175, or 0.25 mg/kg/day but was not dose-dependent. Overall, the findings of the study are of little significance because of severe deficiencies (refer to Section 2.2.2.8 for details).

All reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.7 Genotoxic Effects

One case report was located involving a woman who ingested more than one-half gallon of heptachlor-contaminated milk per day during and after her pregnancy (Chadduck et al. 1987); the level of heptachlor in milk was not provided in the report. Her child was delivered normally and appeared to be healthy. Two weeks after birth, however, the child was diagnosed as having a cerebral gliosarcoma. Cytogenetic analyses of tumor cells revealed chromosomal anomalies including translocations, rearrangements, and breaks. The presence of chromosomal abnormalities suggests the possibility of either environmental or familial causes. However, most tumor cells exhibit abnormal karyotypes. Therefore, heptachlor is only a potential factor in the etiology of the cerebra1 gliosarcoma (Chadduck et al. 1987).

In two dominant lethal studies, neither heptachlor nor heptachlor epoxide proved to be clastogenic in the germ-line cells of male Charles River or Swiss mice (Arnold et al. 1977; Epstein et al. 1972). Mice in one study were given a single oral dose of heptachlor:heptachlor epoxide (25:75) at 7.5 or 15 mg/kg (Arnold et al. 1977). The other study involved five daily oral doses at 5 or 10 mg/kg for heptachlor or 8 mg/kg for heptachlor epoxide (Epstein et al. 1972).

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to heptachlor or heptachlor epoxide.

Dietary administration of heptachlor (97.6% purity) at 0.65 or 1.3 mg/kg/day for 25 weeks promoted the development of hepatocellular foci and hepatocellular neoplasms in male $B6C3F_1$ mice previously initiated with 3.8 mg/kg/day diethylnitrosamine in drinking water for 14 weeks (Williams and Numoto 1984). These results indicate that heptachlor acts as a liver tumor promoter in male mice.

Hepatocellular carcinoma was significantly increased in mice of both sexes following a chronic feeding study in which the mice received technical-grade heptachlor (73%) at 1.8 mg/kg/day heptachlor for males and 2.3 mg/kg/day heptachlor for females for 80 weeks. These were the highest doses for each sex that were

tested. Body weights were similar for exposed animals and controls. However, signs of toxicity including alopecia, rough coats, and palpable masses were seen in both treated and control animals. A dose-related decrease in survival was noted in high-dose females (NCI 1977).

Osborne-Mendel rats were fed technical-grade heptachlor (73%); males received TWA doses of 1.94 and 3.9 mg/kg/day and females received TWA doses of 1.28 and 2.56 mg/kg/day for 80 weeks (NCI 1977). The results of this study showed a statistically significant increase in follicular cell neoplasms in the thyroid (adenomas and carcinomas) in females fed the high dose compared to controls. This finding was discounted by the investigators, however, because the incidence rates were low and are known to be variable in the control rat population. Rates of tumor incidences in males were not increased.

In a 2-year chronic study, dietary exposure of CF rats to heptachlor failed to produce biologically and statistically significant treatment-related effects (Witherup et al. 1955). Six groups of rats (20/sex) were fed diets containing heptachlor at concentrations of 0, 0.038, 0.075, 0.125, 0.175, or 0.25. mg/kg/day. The mortality noted among animals was not dose-dependent and may have been age related or due to ill health since animals developed pneumonia and in some cases hepatitis or other diseases. Daily food intake varied among treated animals, varied from period to period, and displayed no uniformity. Variation in the body weight followed very closely the variation in food consumption, irrespective of the amounts of heptachlor ingested. The occurrence of various types of tumors primarily among dead animals was unrelated to the treatment and may have been spontaneous in origin and age related. The study had several deficiencies including a faulty diet preparation method, improper dose selection, and crude and insensitive methods for evaluation of toxicity. Moreover, it was conducted in the 1950s when test guidelines were not established. There was a lack of dose-response patterns in the reported results, and the concentrations of heptachlor used were too low to produce toxicologically significant effects, which suggests that the maximum tolerated dose was not achieved.

EPA has classified heptachlor and heptachlor epoxide in Group B2 (possible human carcinogen) (IRIS 1990). The International Agency for Research on Cancer (IARC) has classified heptachlor and heptachlor epoxide as Group 3 chemicals (not classifiable as to human carcinogenicity) (IARC 1979).

The Cancer Effect Level (CEL) inmice from chronic exposure to heptachlor is recorded in Table 2-l and plotted in Figure 2-l.

2.2.3 Dermal Exposure

There is very little information on dermal exposures in either humans or animals. Most occupational exposures to heptachlor and heptachlor epoxide are assumed to be some combination of inhalation and dermal exposure, but there are no data to quantitate the relative contribution of each route. The occupational studies on pesticide workers are discussed in Section 2.2.1.

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to heptachlor or heptachlor epoxide.

For heptachlor dissolved in xylene and administered once, Gaines (1969) reported LD_{50} values in Sherman rats of 195 mg/kg (males) and 250 mg/kg (females). Therefore, the dermal LD_{50} for heptachlor in rats is between 195 and 250 mg/kg heptachlor (Ben-Dyke et al. 1970; Gaines 1969). The studies are limited by

the lack of procedural details regarding the vehicle used for administration and the absence of data on the purity of the test compounds.

2.2.3.2 Systemic Effects

No studies were located regarding systemic effects in humans or animals after dermal exposure to heptachlor or heptachlor epoxide.

2.2.3.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to heptachlor or heptachlor epoxide.

2.2.3.4 Neurological Effects

One human case report was located that described confusion and convulsions occurring about 40 minutes after a woman spilled an unknown amount of chlordane on her clothing (Derbes et al. 1955). The woman died shortly after the onset of convulsions; autopsy showed congestion and edema of the brain and scattered petechiae. Technical-grade chlordane contains varying amounts of heptachlor. However, exposure measurements were not provided in the report.

No studies were located regarding neurological effects in animals after dermal exposure to heptachlor or heptachlor epoxide.

No studies were located regarding the following health effects in humans or animals after dermal exposure to heptachlor or heptachlor epoxide:

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to heptachlor or heptachlor epoxide.

2 . 3 TOXICOKINETICS

2.3.1 Absorption

2.3.1 .l Inhalation Exposure

No studies were located regarding absorption in humans after inhalation exposure to heptachlor or heptachlor epoxide.

Although the database is extremely limited, one study has examined inhalation exposure in rabbits under environmental conditions (Arthur et al. 1975). One group of 20 white rabbits (10 male, 10 female; strain not reported) was housed outdoors in an area of high pesticide use, in cages under an aluminum roof allowing free air movement. A second, equal-sized group was housed inside a building in an area of low pesticide use. During the 3-month exposure, weekly air sampling revealed the heptachlor epoxide concentration to be 1.86 ng/m^3 in the high-exposure area. Heptachlor epoxide was not measured in the indoor area and was assumed negligible based on previous low measures of DDT. Respiratory intake of heptachlor epoxide was calculated to be 0.002 μg day; heptachlor epoxide was not detectable in the feed. At the end of the exposure period, serum and fat concentrations of heptachlor epoxide were measured. It was found that heptachlor epoxide in the fat of exposed rabbits was significantly higher than that measured in control animals (0.039-±-0.002 versus 0.016±0.001). No heptachlor epoxide was detected in any serum sample.

2.3.1.2 Oral Exposure

In order to assess the potential extent of human exposures and health effects, members of dairy farm families who consumed raw dairy products known to be contaminated with heptachlor epoxide were studied (Stehr-Green et al. 1986). These individuals and an unexposed urban reference population were compared with regard to serum pesticide levels and liver toxicity. The farm family members had significantly higher mean serum levels of heptachlor epoxide (0.81±0.94 ppb), oxychlordane (0.70±0.75 ppb), and transnonachlor (0.79±.60 ppb) than the unexposed population. This study is limited because exposure level, duration, and frequency of exposure are not known. There was no increase in prevalence of abnormal liver function tests in the dairy farm families compared to the urban population. There are insufficient data to make a quantitative estimate for. absorption of heptachlor in humans following oral exposure.

Heptachlor is absorbed from the gastrointestinal tract of rats (Radomski and Davidow 1953; Tashiro and Matsumura 1978) and cattle (Harradine and McDougall 1986) as indicated by the presence of heptachlor and/or its metabolites in serum, fat, liver, kidney, and muscle (Radomski and Davidow 1953) and by its oral toxicity in several animal species including rats, mice, hamsters, guinea pigs, and rabbits (LD_{50}, 40-162 mg/kg/day) (Ben-Dyke et al. 1970; Eisler 1988; Gaines 1969; Gak et al. 1976; Lehman 1951; Podowski et al. 1979; Sun 1972). Heptachlor epoxide is also absorbed after oral administration to rats (Gillett and Chan 1968). However, no quantitative data that specifically describe absorption of heptachlor epoxide following oral exposure were found in the literature.

Only 6% of the radioactivity from radiolabeled (^{14}C) heptachlor was found in the urine while 60% was found in the feces of male rats 10 days after a single oral dose indicating that most of the radioactive material was not absorbed and was excreted in the feces (Tashiro and Matsumura 1978). These data strongly suggest that a large percentage of heptachlor is absorbed from the gastrointestinal tract and eliminated via the bile into the feces. More than 72% of the radioactivity eliminated in the feces was present as metabolites of heptachlor (heptachlor epoxide, 13.1%; H-2, <0.1%; l-OH-chlordene, 19.5%; l-OH-chlordene epoxide, 17.5%; 1,2-OH-chlordene, 3.5%; H-6, 19.0%).

Three groups of four Australian Hereford steers were placed in a paddock that had been previously treated twice, 3 years earlier, with 0.275 kg heptachlor/hectare (Harradine and McDougall 1986). The mean heptachlor and heptachlor epoxide residues measured in soil samples averaged 0.136 ppm and 0.117 ppm, respectively. Soil samples showed substantial variability about the mean with no relation to date of sampling; this was attributed by the authors to uneven applications of heptachlor to the pasture, resulting

in “hot spots” which were not always sampled. Biopsies of fat from the steers were taken to monitor heptachlor uptake. Within 4 weeks of grazing in the contaminated paddock, one group of animals had heptachlor epoxide present in their body fat at levels that exceeded the Australian maximum residue limit of 0.2 mg/kg. In all groups of cattle, adipose tissue levels of heptachlor epoxide were inversely related to pasture grass length. The authors believe that when pasture grasses are shorter than 50 mm, ruminants ingest a great amount of pasture soil through close grazing, thus accounting for the relationship between pasture grass length and heptachlor and heptachlor epoxide intake. Although this study supports oral absorption of heptachlor and/or heptachlor epoxide in cattle, there are insufficient data to make a quantitative estimate of absorption fraction or absorption rate.

2.3.1.3 Dermal Exposure

No studies were located regarding absorption in humans after dermal exposure to heptachlor or heptachlor epoxide.

Heptachlor is absorbed through the skin following topical application as indicated by its dermal toxicity in rats (LD_{50}, 195-250 mg/kg) (Gaines 1969), but quantitative data are not available. Rats were given heptachlor dissolved in xylene (concentration of heptachlor unspecified), formulated to give a dose at a rate of 0.0016 mL/g body weight. The rats were not restrained and no attempt was made to remove the heptachlor from the shaved area of skin following the exposure, and therefore, some absorption following ingestion may also have occurred.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide.

2.3.2.2 Oral Exposure

No human studies were located regarding the distribution of heptachlor and its metabolites after oral exposure. However, there is an abundance of information reporting heptachlor and heptachlor epoxide in various tissues sampled at autopsy or during surgery, and in serum and milk from humans after exposure via unknown routes. Since the majority of data are from the period when heptachlor was widely used in agriculture, making the ingestion of heptachlor through contaminated agricultural products likely, human tissue, serum, and milk levels are presented in this section. It is possible, however, that other routes of exposure may have contributed to the overall body burden of heptachlor and heptachlor epoxide.

In the human studies described below, levels of organochlorine pesticides were measured in various tissues of adults at autopsy; in stillborn infants and newborns at autopsy; and in body fat, human milk, and serum. With the exception of one study (Stehr-Green et al. 1986), all of the studies are limited by the unknown exposure history of the individuals.

Autopsies of 77 Hawaiian individuals between 1966 and 1968 found heptachlor epoxide in tissues at levels ranging from 1 to 32 ppb (Klemmer et al. 1977). The highest levels of heptachlor epoxide occurred in bone marrow and liver, although the actual levels were not provided in the study. Autopsies of 271 patients with various terminal diseases detected heptachlor epoxide concentrations in fat

(0.21±0.11-0.48±0.37 ppm) and to a lesser degree in liver and brain (trace to 0.05 ppm and trace to 0.01 ppm, respectively) (Radomski et al. 1968). There appeared to be no correlation between the cause of death and the heptachlor epoxide concentration or pesticide usage during the lifetime of the individual.

Heptachlor epoxide was measured in a strip of skin, fat, and subcutaneous tissue from 68 children who died in the perinatal period and ranged from not detected (nondetectable) to 0.563 ppm (mean 0.173) (Zavon et al. 1969). In 10 other stillborn infants, heptachlor epoxide levels measured in various tissues were as follows: brain (nondetectable), lung (0.17±0.07 ppm), adipose (0.32±0.10 ppm), spleen (0.35±0.08 ppm), liver (0.68±0.50 ppm), kidney (0.70±0.28 ppm), adrenal (0.73±.27 ppm), and heart (0.80±0.30 ppm) (Curley et al. 1969). In another study, the following heptachlor epoxide levels were measured in extracted lipids from mothers and newborn infants: maternal adipose tissue (0.28±.31 ppm), maternal blood (0.28±0.46 ppm), uterine muscle (0.49±.51 ppm), fetal blood (1.00±0.95 ppm), placenta (0.50±0.40 ppm), and amniotic fluid C0.67±1.16 ppm) (Polishuk et al. 1977a). These data provide evidence of transplacental transfer to the fetus.

Heptachlor and heptachlor epoxide were measured in 51 human milk samples at average concentrations of 0.0027 and 0.019 ppm, respectively, from women with unknown exposure histories (Jonsson et al. 1977). Heptachlor epoxide was found in 24% of the samples, and heptachlor in 6%. Other investigators have reported the presence of heptachlor epoxide in human milk at concentrations ranging from not detected to 0.46 ppm (Kroger 1972; Polishuk et al. 1977b; Savage et al. 1981; Takei et al. 1983), suggesting a potential for lactational transfer to the fetus.

Unchanged heptachlor has not been detected in human adipose tissue; however, heptachlor epoxide was measured in adipose tissue at levels ranging from 0.0001 to 1.12 ppm (Barquet et al. 1981; Burns 1974; Greer et al. 1980; Radomski et al. 1968; Wasserman et al. 1974) and in plasma at 0.0136±0.0057 ppm (Polishuk et al. 1977b).

Animal studies regarding heptachlor and heptachlor epoxide distribution in body tissues are limited. When 20 adult female rats were fed heptachlor in their diet at a level of 35 ppm for 3 months, examination of the body fat revealed a high concentration of heptachlor epoxide at 3 months but no heptachlor (Radomski and Davidow 1953). Further studies in rats showed that accumulation of heptachlor epoxide was directly related to the dose of heptachlor given. A more detailed examination of the deposition of heptachlor epoxide in body tissues after oral administration under similar exposure conditions showed that the highest concentrations were found in the fat; markedly lower amounts were found in liver, kidney, and muscle; and none was found in the brain. In a parallel study, three dogs were also examined. Doses of 1 mg/kg/day for 12-18 months revealed the same distribution picture as in rats, but the livers of dogs contained more heptachlor epoxide than the kidneys and muscle tissue. Levels in all tissues were higher in female dogs than in males. This is interesting in light of the fact that male rats were more sensitive than female rats to heptachlor toxicity (Gaines 1969), suggesting a species difference.

The rate of heptachlor epoxide accumulation in, and elimination from, fat was determined in rats fed diets containing 30 ppm heptachlor for 12 weeks, then fed untreated diets for 12 more weeks (Radomski and Davidow 1953). Animals were sacrificed at various times during treatment, and it was shown that the residue in the fat of males reached a plateau at approximately 2-8 weeks. Thereafter, the levels decreased and were below the detection limit by the end of week 6 postdosing. In females, the heptachlor epoxide level in fat was much higher than males by the second week and throughout the remainder of the study. By the end of the 8th week postdosing, the heptachlor epoxide was below the detection limit in females.

Heptachlor and heptachlor epoxide residues were found in the fat (≥0.16 ppm and ≥18.25 ppm, respectively), liver (≥0.08 ppm and ≥2.11 ppm, respectively), and muscle (0 and ≥0.03 ppm, respectively) of pigs fed 2 mg/kg/day heptachlorine (purity unspecified) for 78 days (Halacka et al. 1974). When pigs were fed 5 mg/kg/day, the levels of heptachlor and heptachlor epoxide were higher: 0.37 and 25.82 ppm, respectively, in the fat; 0.23 and 4.94 ppm, respectively, in liver; and 0 and 0.7 ppm, respectively, in muscle.

Detection of heptachlor epoxide may indicate either recent or past exposure. This compound has a long half-life, particularly in adipose tissue, because it is very lipophilic. Because of its highly lipophilic nature, heptachlor epoxide remains accumulated in adipose tissue for months to years. However, it is eventually mobilized into the serum and subsequently to the liver for further breakdown. Blood serum levels are often taken to indicate a recent exposure. Following long-term exposure, the level in the blood may be very low, but because of an equilibrium between fat and blood, it can be used to detect exposure to heptachlor epoxide. Thirty-five human adipose tissue samples were obtained during autopsy between 1987 and 1988 from residents of North Texas (Adeshina and Todd 1990). In 97% of these samples, there were measurable levels of heptachlor epoxide that were positively correlated with age for the age groups 41-60 years and 61 and older. No differences between sexes were noted. These results indicate that the tissue levels of heptachlor epoxide in the human population from the above geographical region have not significantly decreased since 1970.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to heptachlor or heptachlor epoxide.

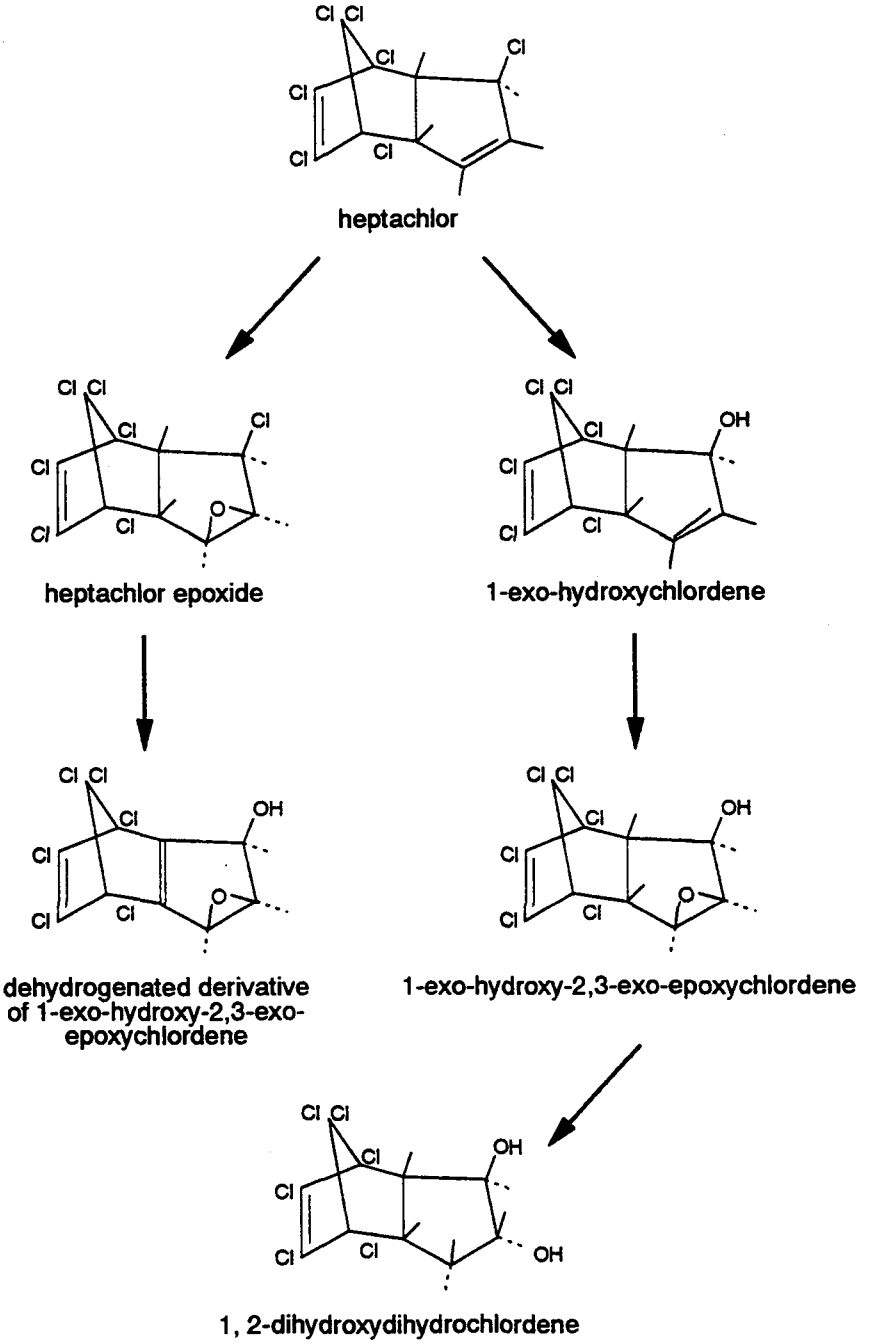
2.3.3 Metabolism

No studies were located regarding metabolism of heptachlor or heptachlor epoxide in humans. However, animal studies have shown that heptachlor undergoes epoxidation to produce heptachlor epoxide, which is more toxic than its parent compound. Heptachlor epoxide is further metabolized and excreted. In an in vitro liver study, human and rat liver microsomes metabolized heptachlor to the same products but in different proportions (Tashiro and Matsumura 1978). It was also shown in this study that rat microsomal preparations were four times more efficient in the metabolic conversion of heptachlor to heptachlor epoxide than were human microsomal preparations.

The major fecal metabolites in male rats administered a single oral dose of ^{14}C-heptachlor are heptachlor epoxide, l-exo-hydroxychlordene,1-exo-hydroxy-2,3-exo-epoxychlordene,and1,2 dihydrovdihydrochlordene, as well as two unidentified products (Figure2-2) (Tashiro and Matsumura 1978). By day 3, 50% of the dose was excreted in the feces. About 72% of the radioactivity was eliminated in the feces in the form of metabolites and 26.2% as parent compound by day 10. The same metabolites were identified in the comparative in vitro study using rat and human microsomal preparations (Tashiro and Matsumura 1978). Heptachlor epoxide is metabolized one step further to a dehydrogenated derivative of l-exo-hydroxy-2,3-exoepoxychlordene. Less than 0.1% of radiolabel was seen of this compound in an in vitro study using human liver microsomes (Tashiro and Matsumura 1978).

Heptachlor is formed through the metabolism of chlordane. Heptachlor epoxide is formed through the epoxidation of heptachlor and has been shown to be a cosubstrate of the same enzyme responsible for the epoxidation of aldrin to dieldrin (Gillett and Chan 1968). Heptachlor epoxide is considered more toxic

FIGURE 2-2. Metabolic Scheme for Heptachlor in Rats*



*** Adapted from Tashiro and Matsumura 1978**

than its parent compound and, like heptachlor, is primarily stored in adipose tissue (Barquet et al. 1981; Burns 1974; Greer et al. 1980; Harradine and McDougall 1986).

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals after inhalation exposure to heptachlor or heptachlor epoxide. Based on the data from oral studies, heptachlor is expected to be excreted primarily in the form of metabolites and also as unchanged parent compound.

2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to heptachlor or heptachlor epoxide.

The elimination of a single oral dose of ^{14}C-heptachlor in male rats showed that most of the radioactivity was eliminated in the feces (Tashiro and Matsumura 1978). One day after dosing, 36% of the dose had been eliminated, and by day 10, approximately 62% had been eliminated in the feces. Elimination of the radioactive label in urine accounted for only 6% of the total dose in 10 days. Approximately 26.2% of the total radioactivity recovered from the feces was the parent compound and the remainder was in the form of metabolites.

Elimination of heptachlor epoxide via milk production was found to maximize within 3-7 days in cows that had grazed on pastures immediately following treatment of the grasses with heptachlor. The level of heptachlor epoxide in the milk was 0.22 ppm (Gannon and Decker 1960).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to heptachlor or heptachlor epoxide. Based on the data from oral studies, heptachlor is expected to be excreted primarily in the form of metabolites and also as unchanged parent compound.

2.4 RELEVANCE TO PUBLIC HEALTH

Although few quantitative data on exposures and measurable adverse health effects exist for humans, there is evidence that heptachlor and heptachlor epoxide can cause adverse effects if exposure is sufficient in duration and/or dose. Heptachlor is one of the cyclodiene pesticides designed to act as a neurotoxicant in insects. It is not surprising, therefore, that the central nervous system can be identified as one of the target systems of this compound in humans and animals. The liver is also a target organ for heptachlor and heptachlor epoxide. The findings of changes in liver enzymes and histopathology in several animal species indicate that the liver would be a target for humans also. There is some evidence from the few metabolic studies available that male rats may be more sensitive than female rats. Interestingly, a study on dogs provided evidence that the livers and other tissues of the females concentrated higher levels of heptachlor epoxide, although no differences in response were noted.

Heptachlor was classified by IARC as having some evidence for carcinogenicity although it tested negative in in vitro tests for deoxyribonucleic acid (DNA) repair (Williams et al. 1989). This evidence against

heptachlor having a direct effect on the DNA molecule is consistent with evidence from other chemicals that some chemicals act as epigenetic carcinogens and produce neoplasia by nongenotoxic mechanisms. Additional analysis based on the structure-activity relationships of 189 chemicals supports the possibility that heptachlor may contain non-electrophilic structures in common with other nongenotoxic carcinogens (Rosenkranz and Klopman 1990).

Death. Occupational mortality studies of pesticide workers exposed to heptachlor have not revealed an excess number of deaths in these cohorts compared to the general U.S. population. This may possibly be explained as a healthy worker effect. The EPA has described human case reports in which convulsions and death were reported following suicidal ingestion of technical-grade chlordane, which typically contains 6-30% heptachlor, but these effects cannot be attributed to heptachlor or heptachlor epoxide. There are no controlled, quantitative human data for any route of exposure. Acute lethality data were located for animals exposed via the oral and dermal routes. Both heptachlor and heptachlor epoxide may be considered very toxic via the oral route on the basis of acute animal data in rats and mice. Intermediate oral exposure to these compounds also caused up to 40% and 100% mortality in rats and mice, respectively. There appear to be differences in sensitivity in males and females in some species with the males being most sensitive. Heptachlor epoxide is more toxic than heptachlor. Heptachlor may be considered very toxic to extremely toxic via the dermal route on the basis of acute lethabty data in rats and mice. The severity of acute effects may possibly depend upon the extent of formation of heptachlor epoxide and the species tested.

Systemic Effects

Cardiovascular Effects. There is evidence to suggest that the effects of heptachlor on the atherosclerotic process are involved in both cardiovascular and cerebrovascular disease. The incidence of cerebrovascular disease was significantly increased in workers engaged in the manufacture of chlordane, heptachlor, and endrin, but was not increased in pesticide applicators and termite control operators thought to have the potential for high-level exposures to chlordane and heptachlor by unspecified routes. These studies were limited because of the lack of control for confounding variables such as preexisting cardiovascular disease and other risk factors such as smoking and dietary habits. There are no animal studies that confirm or refute cardiovascular effects following heptachlor or heptachlor epoxide exposure from any route. The effects of heptachlor on liver function, gluconeogenic enzymes, and steatosis could potentially be involved in the atherosclerotic process. Increases in gluconeogenic enzymes and hepatocyte production of lipids could cause increased serum levels of lipids, which in turn contribute to atherosclerosis.

Hematological Effects. Intermediate and chronic inhalation exposure of humans to mixtures of heptachlor, chlordane, and other chemicals has been associated with leukemia and aplastic and hemolytic anemias. These exposures were either occupational or followed the use of termiticides in homes. These exposures were probably primarily inhalation combined with dermal. There are oral animal studies that confirm that the hematopoietic system, specifically the white cells, can be affected by heptachlor exposure. Rats fed 0.5 mg/kg/day heptachlor in the diet showed a statistically significant increase in total white blood count (Enan et al. 1982). It appears that although the hematopoietic system is not a primary target for heptachlor or heptachlor epoxide, it can be measurably affected.

Hepatic Effects. There are a few epidemiological studies that have attempted to identify hepatic changes in humans exposed primarily via the inhalation route to heptachlor; so far these have been negative. On the basis of animal data, hepatotoxicity may be the most sensitive systemic end point for heptachlor and heptachlor epoxide; signs of toxicity in animals following short- or long-term oral exposure include

histologic evidence of liver damage, a statistically significant increase in liver weight, and increased levels of serum enzymes such as alanine aminotransferase (ALT), glutamine aminotransferase (GLT), and lactate dehydrogenase (LDH) indicative of hepatic damage. Decreased body weight gain has often been reported in conjunction with the induction of hepatotoxicity by intermediate or chronic oral exposure to heptachlor or heptachlor epoxide. Although these animal studies have limitations in either design or conduct, the hepatic effects seen are generally consistent across species. Heptachlor also induces cytochrome P-450 enzymes, which in turn aid the metabolism of heptachlor to heptachlor epoxide, a more toxic product. This, in effect, constitutes "self" bioactivation.

There are data from animal studies in mice, rats, and pigs that indicate that both carbohydrate metabolism and lipid metabolism may be affected by exposure to heptachlor or heptachlor epoxide (Enan et al. 1982; Halacka et al. 1974; Kacew and Singhal 1973, Pelikan 1971). Alterations in gluconeogenic enzymes and an increase in cellular steatosis in the liver have been reported. Granulomas and fibrotic liver have also been observed. In addition, hepatocellular carcinoma was identified as causally related to heptachlor in the diet in a mouse study conducted by the National Cancer Institute (NCI 1977). The existing evidence suggests that heptachlor and heptachlor epoxide are hepatic toxicants.

Chronic intramuscular injection of rats with heptachlor, heptachlor epoxide, or endrin (cyclodiene compounds) for 45 consecutive days significantly elevated the concentration of blood glucose and the levels of liver pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose l,Bdiphosphatase, and glucose 6-phosphatase. In addition, a significant decrease in hepatic glycogen content was noted in the animals receiving either of the three cyclodiene compounds (Kacew et al. 1973). However, in vitro studies of heptachlor epoxide in mouse liver homogenates showed no effects on enzyme succinic dehydrogenase activity at molar concentrations of $0.166x10^{-5}$, $0.332x10^{-5}$, $1.66x10^{-5}$, and $3.32x10^{-5}$ (Gasper and Kawatski 1972). At a molar concentration of $1.66x10^{-5}$, heptachlor epoxide caused slight inhibition of the enzyme system.

Renal Effects. There are some data that provide evidence of renal effects (uremia, nephrosis) in humans after deliberate oral exposure to heptachlor in chlordane. Target organ toxicities observed in rats and mice during long-term oral exposures include renal effects. Intramuscular injection with heptachlor, heptachlor epoxide, or endrin in rats for 45 consecutive days significantly elevated the concentration of blood urea and increased gluconeogenic enzyme activity in the kidney cortex (Kacew et al. 1973). While these enzyme changes do not necessarily indicate toxicity, they do indicate that heptachlor exposure may affect renal function. Granulomas of the kidney have also been associated with oral heptachlor exposure in mice. There are no extensive histopathologic data, but the human and animal data are consistent in the presence of renal effects.

Other Systemic Effects. Adrenal fibrosis with lipid accumulation was reported in one study in mice, but these effects have not been observed in humans known to be exposed to heptachlor and have not been verified in other species. There has been no measurement of adrenal hormone in exposed humans or animals. Body weight changes have, in general, been accompanied by a decrease in food consumption, due possibly to taste aversion.

Neurological Effects. In human case studies, signs of neurotoxicity (irritability, salivation, lethargy, dizziness, labored respiration, muscle tremors, and convulsions) were reported following exposure (route not specified) of humans to technical-grade chlordane, which contains between 6% and 30% heptachlor. These effects cannot, however, be attributed solely to heptachlor (Dadey and Kammer 1953). Neurotoxic signs, including tremors, convulsions, ataxia, and changes in EEG patterns, have been induced in animals

by chronic oral intake of heptachlor and heptachlor epoxide (Formanek et al. 1976). Studies in rat brain suggest that the neurotoxic effects of heptachlor or heptachlor epoxide may involve, in part, (1) interference with nerve action or release of nemotransmitters as the result of inhibition of either Na^{+}-K^{+} ATPase or Ca^{2+}-Mg^{2+} ATPase activity (inhibition of this enzyme results in reduction of Ca^{2-} binding capacity) (Yamaguchi et al. 1979), or (2) inhibition of the function of the receptor for γ-aminobutyric acid (GABA) (Yamaguchi et al. 1980). Because heptachlor was designed to be an insect neurotoxicant, it is not surprising that the central nervous system is a primary target for this chemical. These results could explain the neurotoxic effects observed in humans exposed -to chlordane, which may be partially attributed to heptachlor content.

Neurological damage following exposure to heptachlor and heptachlor epoxide was also observed in young calves. Central nervous system stimulation was manifested early by muscle spasms in the neck and head. These spasms progressed posteriorly and increased in severity, resulting in convulsions and finally death (Buck et al. 1959). The level of intake influenced the amount of heptachlor epoxide storage in the body fat. Heptachlor epoxide was 10 times as toxic to young calves as technical-grade heptachlor. The maximum nontoxic oral dose of heptachlor epoxide was l-2.5 mg/kg compared with 15-25 mg/kg for heptachlor. The authors characterized these symptoms as typical of those produced by other cyclodiene chlorinated hydrocarbon insecticides.

Cyclodiene insecticides produce intense nerve excitation in both vertebrate and invertebrate species (Matsumura 1985; Matsumura and Tanaka 1984). It has been suggested that the biochemical mechanisms by which these chemicals induce hyperexcitation in the central nervous system are due to the release of neurotransmitters caused by the interactions of the insecticide with the picrotoxinin receptor.

Developmental Effects. Heptachlor epoxide was detected in tissues of stillborn infants (Curley et al. 1969). A negative study was conducted in women of child-bearing age who ingested heptachlor-contaminated milk (Al-Omar et al. 1986). However, the authors did not examine or monitor developmental effects in the infants. The resulting data from the above studies were considered inadequate to establish a relationship between exposure to heptachlor and human developmental toxicity. A large cohort of births was investigated in Oahu, Hawaii, following more than a year of heptachlor-contaminated milk consumption by the mothers.. No evidence of an increase in the incidence of malformations was observed in the study population when" compared to equivalent cohorts from other islands of Hawaii and the general U.S. population (Le Marchand et al. 1986). These studies suggest that heptachlor can cross the placenta; in addition, heptachlor epoxide has been detected in breast milk.

Cataracts (Mestitzova 1967) and decreased postnatal survival (Green 1970) were reported in the progeny of rats fed diets containing heptachlor in intermediate- and chronic-duration studies. Data were insufficient to further evaluate these studies. Although the authors did not offer a mechanism, they did rule out vitamin B deficiency in the development of the cataracts. Because cataracts have also been observed in adult rats following oral exposure to heptachlor, there is reason to question whether cataracts actually are a developmental effect. No data were located for other routes of exposure in animals.

There are no data available that suggest that heptachlor or heptachlor epoxide are developmental toxicants at the levels measured in human populations.

Reproductive Effects. The existing data in humans are insufficient to establish a causal relationship between premature delivery and higher levels of heptachlor epoxide found in pregnant women (Wassermann et al. 1982). Because the ascertainment was based on premature delivery and other risk factors were not

controlled, it is not possible to relate the levels found in these women to those seen in the general population.

Following oral or intraperitoneal administration of heptachlor or heptachlor epoxide to male mice that were then bred with untreated females, the preimplantation losses and resorptions were within control limits (Arnold et al. 1977; Epstein et al. 1972). However, lack of corpora lutea counts may have resulted in inaccurate identification of preimplantation losses. On the other hand, when both sexes of mice or rats were fed diets containing heptachlor in multigeneration studies, resorptions were increased relative to controls, and fertility was markedly decreased (Green 1970), in some instances to zero (Akay and Alp 1981). These results seem to suggest that heptachlor affects the female reproductive system and/or the fetuses and may also affect the male reproductive system. No studies were found in which only female rodents were dosed.

Genotoxic Effects. No conclusive data exist to suggest that either heptachlor or heptachlor epoxide are genotoxic to humans. Only one case study was located that reported a possible link between prenatal heptachlor exposure and the chromosomal anomalies associated with an infant gliosarcoma (Chadduck et al. 1987). However, hereditary factors are also possible in this case. Human SV-40 transformed fibroblasts were exposed to heptachlor and heptachlor epoxide in an in vitro study (see Table 2-2). An increase in unscheduled DNA synthesis (UDS) was observed for both chemicals only in the presence of metabolic activators (Ahmed et al. 1977). According to this investigation, certain metabolites of heptachlor may be the genotoxic agents.

There are very few in vivo genotoxicity studies. Only two in vivo studies were located, and both assessed the dominant lethal effects. The results were negative for both studies, implying that neither heptachlor nor heptachlor epoxide are genotoxic to the germ-line cells of male mice when tested alone or as a mixture (Arnold et al. 1977; Epstein et al. 1972). Refer to Table 2-3 for a summary of these results of in vivo studies.

Most of the research regarding the genotoxicity of heptachlor and heptachlor epoxide comes from in vitro studies. The majority of these studies suggest that neither heptachlor nor heptachlor epoxide are genotoxic. One Salmonella typhimurium Ames assay reported gene mutation in the presence of metabolic activators (Gentile et al. 1982). The remaining gene mutation studies involving prokaryotic organisms reported negative responses both with and without metabolic activation (Glatt et al. 1983; Marshall et al. 1976; NTP 1987; Probst et al. 1981; Zeiger et al. 1987). Another prokaryotic study investigated heptachlor's capacity to cause DNA damage. Both S. tynhimurium and Escherichia coli were tested, and the results were negative for both bacteria (Rashid and Mumma 1986). However, since metabolic activators were not employed, it is impossible to know whether or not metabolites of heptachlor would have damaged DNA In fungi, Saccharomvces cerevisiae was negative for gene conversion following heptachlor exposure with and without activation (Gentile et al. 1982), and Aspergillus nidulans was negative for both gene mutation and chromosome malsegregation following exposure to heptachlor epoxide (Crebelli et al. 1986). Metabolic activators were again not utilized with A. nidulans. In vitro studies for mammalian species show mixed results. Rat, mouse, and hamster hepatocytes were negative for UDS (Maslansky and Williams 1981; Probst et al. 1981). Heptachlor without metabolic activation reportedly caused gene mutations in mouse lymphoma cells but not in adult rat liver cells (Telang et al. 1982). Chromosomal aberrations were observed in Chinese hamster ovary cells following exposure to heptachlor with metabolic activation; sister chromatid exchange was also observed both with and without metabolic activation (NTP 1987). Refer to Table 2-2 for a further summary of these results.

TABLE 2-2. Genotoxicity of Heptachlor and Heptachlor Epoxide In Vitro

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (histidine reversion)[a]	Gene mutation	-	-	Zeiger et al. 1987
S. typhimurium (Ames assay)[b]	Gene mutation	-	-	Marshall et al. 1976; NTP 1987
S. typhimurium (Ames assay)[a]	Gene mutation	+	-	Gentile et al. 1982
S. typhimurium (modified Ames assay)[a]	Gene mutation	-	-	Probst et al. 1981
S. typhimurium (modified Ames assay)[c]	Gene mutation	-	-	Glatt et al. 1983
Escherichia coli (modified Ames assay)[a]	Gene mutation	-	-	Probst et al. 1981
S. typhimurium (disc assay)[a]	DNA damage	No data	-	Rashid and Mumma 1986
E. coli (DNA repair assay)[a]	DNA damage	No data	-	Rashid and Mumma 1986
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae (ade, trp loci assay)[a]	Gene conversion	-	-	Gentile et al. 1982
Aspergillus nidulans (strain 35/liquid medium)[c]	Gene mutation	No data	-	Crebelli et al. 1986
A. nidulans (strain P1/liquid medium)[c]	Chromosome malsegregation	No data	-	Crebelli et al. 1986

TABLE 2-2 (Continued)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Mammalian cells:				
Mouse (L5178Y tk^+/tk^- lymphoma cell forward mutation assay)[a]	Gene mutation	No data	+	McGregor et al. 1988
Rat (ARL-HGPRT assay)[a]	Gene mutation	–	NA	Telang et al. 1982
Chinese hamster (ovary cells)[a]	Chromosomal aberrations	+	–	NTP 1987
Chinese hamster (ovary cells)[a]	Sister chromatid exchange	+	+	NTP 1987
Rat (primary hepatocytes)[a]	Unscheduled DNA synthesis	–	NA	Probst et al. 1981
Rat (primary hepatocytes)[a]	Unscheduled DNA synthesis	–	NA	Maslansky and Williams 1981
Mouse (primary hepatocyte)[a]	Unscheduled DNA synthesis	–	NA	Maslansky and Williams 1981
Syrian hamster (primary hepatocytes)[a]	Unscheduled DNA synthesis	–	NA	Maslansky and Williams 1981
Human (SV-40 transformed fibroblasts)[b]	Unscheduled DNA synthesis	+	–	Ahmed et al. 1977

[a]Tested effects of heptachlor only
[b]Tested effects of both heptachlor and heptachlor epoxide individually; result applies to both compounds.
[c]Tested effects of heptachlor epoxide only

– = negative result; + = positive result; ade = adenine; ARL = adult rat liver epithelial cell line; DNA = deoxyribonucleic acid; HGPRT = hypoxanthine-guanine phosphoribosyl transferase; NA = not applicable; tk = thymidine kinase locus; trp = tryptophan

TABLE 2-3. Genotoxicity of Heptachlor and Heptachlor Epoxide In Vivo

Species (test system)	End point	Results	Reference
Mammalian cells:			
CD-1 mouse (dominant lethal assay)	Dominant lethal	-[a,b]	Arnold et al. 1977
Swiss mouse (dominant lethal assay)	Dominant lethal	-[a,c]	Epstein et al. 1972

[a]Result applies to both oral and intraperitoneal routes of exposure.
[b]Heptachlor/heptachlor epoxide mixture (25:75) was used.
[c]Results reflect separate exposures to both heptachlor and heptachlor epoxide.

- = negative result

Several studies were located involving heptachlor genotoxicity in plants. A positive response was noted for the waxy gene mutation in maize (Zea mavs) following exposure to heptachlor in situ (Gentile et al. 1982). A micronucleus test in Tradescantia produced a significant positive dose-related response at 1.88 ppm heptachlor (Sandhu et al. 1989). This study suggests that heptachlor has clastogenic potential in plants. Two researchers conducted a series of studies to determine the effects of certain pesticides (heptachlor included) on mitotic and meiotic chromosomes in Lens culinaris, Lens esculenta, Pisum sativum, and Pisum arvense (Jain and Sarbhoy 1987a, 1987b). For the mitotic segment, positive responses were observed after heptachlor treatment for the following chromosomal abnormalities: early separation during metaphase, condensation, stickiness, and chromatin bridges (Jain and Sarbhoy 1987a). For the meiotic study, heptachlor reportedly caused such chromosomal abnormalities as stickiness, non-orientation during metaphase I, fragments, multivalents, and bridges (Jain and Sarbhoy 1987b). These studies by Jain and Sarbhoy report no statistical comparisons with which to interpret the results; therefore, it is difficult to evaluate the significance of their research. Even though these plant studies suggest that both heptachlor and heptachlor epoxide are potentially genotoxic, the applicability to mammalian genotoxicity remains questionable.

Cancer. Existing epidemiological studies on heptachlor are considered inadequate to establish a clear qualitative or quantitative assessment of heptachlor exposure and human risk of developing cancer. The large occupational cohort mortality studies conducted on workers engaged in the manufacture of heptachlor have not identified a statistically significant increase in cancer deaths. Chronic oral exposure to heptachlor and heptachlor epoxide increased the incidence of liver carcinomas in CFN rats and C3H, CD-l, and $BGC3F_1$, mice. Heptachlor and heptachlor epoxide are classified as possible human carcinogens, Group B2, under EPA's guidelines for carcinogen risk assessment based on the positive cancer findings in animal studies. Heptachlor and heptachlor epoxide are classified as Group 3 by IARC. A Group 3 classification indicates that it is not possible at present to determine the human carcinogenicity of these compounds.

Heptachlor appears to be a promoter of hepatocarcinogenesis in mice. Consistent with this finding, low concentrations of heptachlor inhibited intercellular communication in Chinese hamster cells and rat liver cells, a property common to many known promoters (Williams and Numoto 1984). Of note was the demonstration of assay specificity for detection only of agents that interfere with cell-to-cell communication (epigenetic effect), as opposed to chemicals that induce a genotoxic effect. Overall, therefore, it may be postulated that heptachlor acts .through an epigenetic mechanism rather than one that is genetic.

In vitro treatment of human myeloblastic leukemia ML-l cells with low concentrations of heptachlor (<30 nmol) induced them to differentiate into monocyte and macrophage-like cells (Chuang et al. 1991). These cell types resemble those produced after treatment with 12-0-tetradecanoylphorbal-13-acetate (TPA), a known tumor promoter. Similar to TPA, heptachlor has been shown to inhibit intracellular communication between cultured liver cells (Telang et al. 1982). Based on these similarities, it is speculated that heptachlor and TPA may act by a common mode of action and that heptachlor acts not as a chemical mutagen, but as a tumor promoter (Chuang et al. 1991).

Most of the evidence from genotoxicity assays indicates that neither heptachlor nor heptachlor epoxide act directly on the DNA molecule. The exact mechanism by which these chemicals produce their effects remains unclear, but several lines of investigation are being pursued. Both chlordane and heptachlor have been shown to be potent inducers of protein kinase C activity in both rat and mouse brain. Several other chlorinated hydrocarbons were also positive for this effect; chlordane was the most potent of this set of chemicals (Moser and Smart 1989).

Other work has indicated that chlordane and heptachlor are energy transfer inhibitors as evidenced by marked decreases in oxidative phosphorylation of rat hepatic mitochondria following in vitro incubation of the mitochondria with the pesticides (Ogata et al. 1989). Interestingly, even though heptachlor epoxide is more toxic than either chlordane or heptachlor in tests of general toxicity, it was less effective in inhibiting mitochondrial respiration.

Heptachlor, chlordane, and endosulfan (another cyclodiene pesticide) were shown to inhibit hepatocyte gap junctional interceliular communication (Ruth et al. 1990). All three pesticides showed similar doseresponse relationships. Further testing with chlordane and heptachlor indicated that inhibition of the cytochrome P-450 system had no effect on this response. These results suggest that the interference with intercellular communication is not directly tied into the effects of these cyclodienes on the P-450 system.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NASNRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NASNRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to heptachlor and heptachlor epoxide are discussed in Section 2.51.

Biomarkers of effect are defined as .any measurable biochemical, physiologic, or other alteration within an organism that, depending *on* magnitude, can be recognized as an esrablished or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by heptachlor/ heptachlor epoxide are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2. HEALTH EFFECTS

2.5.1 Biomarkers Used to identify or Quantify Exposure to Heptachlor and Heptachlor Epoxide

Extremely sensitive analytical methods have been developed for the detection of heptachlor and heptachlor epoxide in various environmental and biological samples (detection limits as low as 10 ng/L). Although most methods were developed for detecting heptachlor and heptachlor epoxide in environmental media, the technology is readily adaptable to biological materials including breast milk, adipose tissue, and serum. These methods can be used to determine whether exposure has occurred. The presence of heptachlor may reflect an exposure to heptachlor or chlordane because it is a metabolite of chlordane. The presence of heptachlor epoxide may reflect an exposure to heptachlor or to chlordane since it is a metabolite of both these pesticides. However, in the absence of stable chlordane residues (e.g., nonachlor and oxychlordane), the heptachlor epoxide would most likely have been derived from heptachlor.

Detection of heptachlor or heptachlor epoxide may indicate either recent or past exposure. Heptachlor epoxide has a long half-life, particularly in adipose tissue, because it is very lipophilic and can remain for months to years. However, it is eventually mobilized into the serum and subsequently to the liver for further breakdown. Blood serum levels are often taken to indicate a more recent exposure, but heptachlor epoxide does become mobilized into the serum after being stored in adipose tissue for substantial periods. Thirty-five human adipose tissue samples were obtained during autopsy between 1987 and 1988 from residents of north Texas. In 97% of these samples, there were measurable levels of heptachlor epoxide that were positively correlated with age for the age groups 41-60 years and ≥61 years. No differences between sexes were noted. These results indicate that levels of heptachlor epoxide in human tissues from this region have not significantly decreased since 1970 (Adeshina and Todd 1990).

Pesticide residues were analyzed in 183 milk samples from 165 Finnish women. Heptachlor was found in 12% of the samples; heptachlor epoxide was found in 6.6%. Five percent of the samples contained levels of heptachlor epoxide in excess of 0.0005 mg/kg body weight, an acceptable daily intake (Mussalo-Rauhamaa et al. 1988). Fifteen milk and fat specimens from residents of Grand Forks, British Columbia, and 16 milk and 17 fat specimens from residents of Prince George, British Columbia, were analyzed for pesticide residues. Heptachlor epoxide was found in one milk sample and nine fat samples in the Grand Forks group (>0.004 ppm) and in no milk samples and two fat samples in the Prince George group (>0.004 ppm) (Larsen et al. 1971). The residue was not detectable at levels lower than 0.004 ppm because of limitations of the analytical methods and faulty techniques. It is possible that the potential exposure of the residents to heptachlor may also have occurred via food contaminated with heptachlor.

Organochlorine insecticide residues were determined in samples of human milk, evaporated milk, and prepared baby formulas from various regions of Canada (Ritcey 1972). A mean concentration of 0.003 mg/kg of heptachlor epoxide was detected in human milk, with significantly lower levels in evaporated milk and prepared baby formulas.

No studies were found correlating levels to which humans were exposed with actual body burdens. However, an attempt was made to correlate blood levels of chlordane, which may contain from 6% to 30% heptachlor, to duration of occupational exposure. Blood samples from 51 male pest control operators who were occupationally exposed to chlordane were tested for the presence of chlordane and its metabolites trans-nonachlor, oxychlordane, and heptachlor epoxide. The blood of 19 male workers with no experience : spraying chlordane was also tested as a control. Heptachlor epoxide was detected (from not detectable to 1.6 ppb) in 20% of the blood samples from pest control operators exposed to chlordane (concentration not reported). The total chlordane in the blood was low but demonstrated sizable correlation with the number of spraying days and the amount of chlordane sprayed (Saito et al. 1986).

2.5.2 Biomarkers Used to Characterize Effects Caused by Heptachlor and Heptachlor Epoxide

No specific tests for the effects of heptachlor or heptachlor epoxide were found. The neurological and hepatic effects seen from heptachlor and heptachlor epoxide exposure are typical of exposure to other chlorinated pesticides. An attempt was made to correlate blood residues of heptachlor epoxide to sperm count in a group of 29 infertile men and 14 controls matched for age and smoking (Pines et al. 1987). No correlation could be shown, however. Heptachlor epoxide was found in the blood of 7 out of 39 subjects who drank raw milk contaminated with heptachlor at concentrations as high as 89.2 ppm (fat basis) and in the blood of 3 out of 79 controls (Stehr-Green et al. 1988). The exposed group had significantly higher mean levels of heptachlor epoxide (0.84 ppb) compared to the control group (0.50 ppb). However, no evidence of related hepatic effects in the exposed subjects could be identified. In addition, the study authors were unable to identify a relationship between pesticide levels and dairy fat consumption. The levels of heptachlor found in the milk of four Iraqi women ranged from nondetectable to less than 1 ppm (Al-Omar et al. 1986). No health effects could be associated with these levels.

Although there are no data from human studies that indicate that hepatic effects occur in humans exposed to heptachlor, the animal studies indicate that the liver is a target organ for this chemical and is more sensitive to low doses than the neurological system. Decreased glycogen, increased cholesterol, GOT, and AP enzyme levels, and increased liver weight were reported in mice fed heptachlor at 0.5 mg/kg/day. In contrast, neurological effects such as convulsions were observed in a cow fed 2.5 mg&/day heptachlor daily for 15 days (Buck et al. 1959). Increased liver enzymes could indicate exposure to heptachlor, but this would not be a marker specific to this chemical. Refer to Section 2.2 for a detailed discussion of the effects caused by heptachlor and heptachlor epoxide.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Dietary administration of heptachlor (97.6% purity) at 0.65 or 1.3 mg/kg/day in diet for 25 weeks promoted the development of hepatocellular foci and hepatocellular neoplasms in male B6C3F mice previously initiated with 3.8 mg/kg/day diethylnitrosamine given in the drinking water for 14 weeks (Williams and Numoto 1984).

Nutritional factors may influence the toxicity of pesticides. Research in this area has primarily focused on the role of dietary proteins, particularly sulfur-containing amino acids, trace minerals, and vitamins A, C, D, and E. Studies in rats show that inadequate dietary protein enhances the toxicity of most pesticides but decreases, or fails to affect, the toxicity of a few. ‘The results of these studies have shown that at oneseventh or less normal dietary protein, the hepatic toxicity of heptachlor is diminished as evidenced by fewer enzyme changes (Boyd 1969; Shakman 1974). The lower-protein diets may decrease metabolism of heptachlor to heptachlor epoxide.

Male weanling rats were fed a 5%, 20%, or 40% casein diet for 10 days and then given heptachlor intraperitoneally. The animals receiving the 5% casein diet showed a three-fold tolerance to heptachlor toxicity, but the toxicity of heptachlor epoxide was not affected (Weatherholtz et al. 1969). This was probably due to inability of weanling rats to metabolically convert heptachlor to the more toxic heptachlor epoxide. This fact is further supported by the observation that changes in protein percentage in diet did not affect the toxicity of heptachlor epoxide itself.

Walter Reed-Wistar and Charles River male adult rats were exposed to oral doses of turpentine or to turpentine vapors, which consisted of ∝- and β-pinene. These exposures were followed by oral

administration of heptachlor epoxide or of one of three pesticides, paraoxon, heptachlor, or parathion, or by an intraperitoneal injection of hexobarbital. The studies revealed that pretreatment with turpentine reduced hexobarbital sleeping time, reduced the parathion LD_{50}, and increased the heptachlor LD_{50}. The paraoxon and heptachlor epoxide LD_{50} values were unchanged. α-Pinene and ß-pinene vaporized from turpentine had no effect on either hexobarbital sleeping time or parathion, paraoxon, or heptachlor epoxide mortality but did increase the heptachlor LD_{50} (Sperling et al. 1972). The authors speculated that increases in hepatic microsomal enzyme activity are responsible for these differences.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to heptachlor and heptachlor epoxide than will most persons exposed to the same level of heptachlor and heptachlor epoxide in the environment. Reasons include genetic make-up, developmental stage, health and nutritional status, and chemical exposure history. These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect that the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, "Populations With Potentially High Exposure."

No studies were located indicating that any populations are unusually susceptible to heptachlor or heptachlor epoxide. There is a possibility that very young children may exhibit particular susceptibility to hepatic effects because of the immaturity of the hepatic microsomal system. Heptachlor is bioactivated to produce heptachlor epoxide which is more toxic than heptachlor. Pre-adolescent children have a greater rate of glutathione turnover, and they are expected to be more susceptible to heptachlor epoxide–induced toxicity. Their susceptibility would probably depend upon their ability to detoxify heptachlor epoxide. Individuals who show reduced liver function for other reasons, such as glutathione deficiency, might also be unusually susceptible (Calabrese 1978). However, Harbison (1975) observed that heptachlor was less toxic in newborn rats than in adult rats. Newborn rats pretreated with phenobarbital were more sensitive to the effects of heptachlor than those not pretreated. Thus, the ability to metabolize and bioactivate heptachlor correlates with its toxicity in the newborn. The difference in blood heptachlor epoxide levels among Asians and U.S. residents (Rodomski et al. 1971b) may suggest the involvement of a genetic factor in the susceptibility to heptachlor epoxide toxicity.

There is some evidence in laboratory animals that high-protein diets cause more rapid conversion of heptachlor to heptachlor epoxide and therefore increase the toxicity resulting from exposure to heptachlor. The lack of corroborating data in humans on this phenomenon, however, makes it difficult to postulate that high- or very high–protein diets would significantly increase susceptibility to heptachlor toxicity.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to heptachlor and heptachlor epoxide. However, because some of the treatment discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to heptachlor or heptachlor epoxide. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Human exposure to heptachlor or heptachlor epoxide can occur by inhalation, oral, or dermal contact. Treatment of exposure to these substances is primarily supportive. Following a significant inhalation exposure, the patient is removed from the source to fresh air. Treatment may include administering oxygen and, if needed, maintaining ventilation with artificial respiration (Stutz and Janusz 1988; Bronstein and Currane 1988). General recommendations for reducing absorption of heptachlor following acute dermal exposure have included removal of contaminated clothing followed by washing the skin and hair with soap and water, then with alcohol, then again with soap and water (HSDB 1992; Morgan 1982; Stutz and Janusz 1988). Since leather absorbs pesticides, it has been recommended that leather not be worn while using heptachlor or heptachlor epoxide, and that any leather contaminated with these substances be discarded (HSDB 1992). Oils have not been recommended as dermal cleansing agents because they could increase absorption (Haddad and Winchester 1990). If the eyes have been exposed, they are flushed with water (Bronstein and Currance 1988; Stutz and Janusz 1988). Treatment for ingestion of this substance may require gastric emptying by gastric lavage (Haddad and Winchester 1990) and administration of activated charcoal and cathartic (HSDB 1992; Morgan 1982; Stutz and Janusz 1988; Haddad and Winchester 1990). Heptachlor may be present with a hydrocarbon vehicle which could result in aspiration pneumonitis following the induction of emesis. Therefore, emesis may not be indicated. Some sources do not recommend the use of emetics (Bronstein and Currance 1988), although others do under some circumstances (HSDB 1992; Morgan 1982; Stutz and Janusz 1988). Treatments such as emesis and lavage may be most appropriate following ingestion of large quantities; it is unlikely that the types of exposure likely to occur at hazardous waste sites would require such measures. Treatment with milk, cream, or other substances containing vegetable or animal fats, which enhance absorption of chlorinated hydrocarbons, has not been recommended (Haddad and Winchester 1990; Morgan 1982). If seizures occur, diazepam administration, followed if necessary by additional anticonvulsant medicines such as phenytoin, pentobarbital, thiopental, or succinylcholine, may be recommended (Bronstein and Currance 1988; HSDB 1992; Morgan 1982; Stutz and Janusz 1988). As adrenergic amines, such as epinephrine, may further increase myocardial irritability and produce refractory ventricular arrhythmias, their use has not been recommended (Bronstein and Currance 1988; HSDB 1992; Morgan 1982; Haddad and Winchester 1990).

2.8.2 Reducing Body Burden

Heptachlor is rapidly metabolized by the body, mostly to heptachlor epoxide. Most of the metabolites are rapidly excreted in the feces, with the adipose tissue serving as the major storage depot for the remainder. From the fat, heptachlor epoxide can be slowly released into the bloodstream for further metabolism and excretion. Cholestyramine resin may accelerate the biliary-gastrointestinal excretion of the more slowly eliminated organochlorine compounds, and its use has been suggested (Morgan 1982). Because of the lipophilicity of heptachlor and heptachlor epoxide, dialysis and exchange transfusion are thought to be ineffective (HSDB 1992).

Because heptachlor epoxide is lipophilic, it is likely that the loss of adipose tissue, as may occur during fasting, will mobilize the stored compound and increase the rate of its elimination. However, this mobilization is also likely to temporarily increase the blood levels of heptachlor epoxide. Hence, any possible benefits due to a reduced body burden accompanying fat reduction would need to be balanced against potential harmful results due to the expected temporary increase in blood levels.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

Since the metabolized form of heptachlor, heptachlor epoxide, is the most toxic, it may be possible to reduce the toxic effects of heptachlor by inhibiting the enzyme catalyzing this conversion. This is the same enzyme that catalyzes the epoxidation of aldrin to dieldrin (Gillett and Chan 1968). Further research into the specificity of this enzyme, drugs that could inhibit the enzyme, and any side effects of these drugs could help to determine the feasibility of such a treatment strategy.

In the central nervous system, symptoms observed in animals following exposure include tremors, convulsions, ataxia, and changes in EEG patterns (Formanek et al. 1976). These central nervous system symptoms could be due either to (1) inhibition of the Na^+/K^+ ATPase or the Ca^+/Mg^+ ATPase activity, which can then interfere with nerve action or release of neurotransmitters (Yamaguchi et al. 1979) and/or (2) inhibition of the function of the receptor for GABA (Yamaguchi et al. 1980). In support of the latter possibility, another study showed that heptachlor epoxide inhibited the GARA-stimulated chloride uptake in the coxal muscle of the American cockroach and directly competed against [^{3}H]a-dihydropierotoxinin for binding in the rat brain synaptosomes. These results indicate that some of the nerve excitation symptoms that insecticides cause are probably due to their interaction with the picrotoxinin receptor (Matsumura and Ghiasuddin 1983). A more detailed understanding of the mechanism of heptachlor/heptachlor epoxide action on the central nervous system may lead to new approaches for reducing the toxic effects.

2.9 ADEQUACY OF THE DATABASE

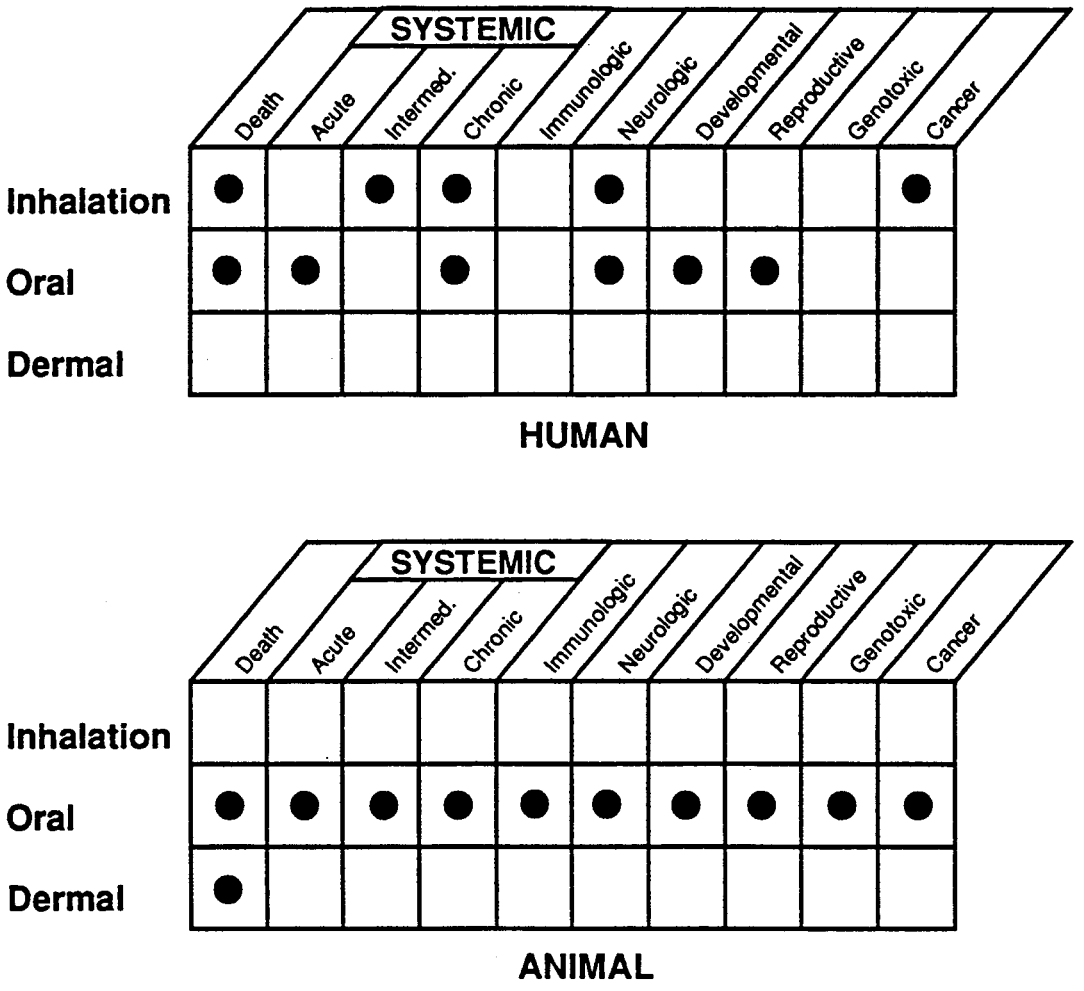
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of heptachlor and heptachlor epoxide is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of heptachlor and heptachlor epoxide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessmen*t.* This definition should *not* be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing information on Health Effects of Heptachlor and Heptachlor Epoxide

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to heptachlor and heptachlor epoxide are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of heptachlor and heptachlor epoxide. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs” information (i.e., data gaps that must necessarily be filled).
Most of the data located concerning the health effects of heptachlor and heptachlor epoxide in humans come from case reports and occupational epidemiology studies of workers engaged either in the

FIGURE 2-3. Existing Information on Health Effects of Heptachlor and Heptachlor Epoxide



● Existing Studies

manufacture or application of pesticides. There is. some information on people who have consumed heptachlor-contaminated food or dairy products, but no adverse health effects have been related to these exposures. The occupational studies involve exposures that are predominantly inhalation with contributions from dermal exposure, whereas all the animal studies were conducted using oral or intraperitoneal exposures. The occupational and case reports provide no quantitation of dose or duration of exposure, which makes it impossible to determine with any precision the effect levels for humans. There are no data that indicate that heptachlor or heptachlor epoxide are carcinogenic to humans. However, human studies are limited by the long latency period of carcinogenesis and by ascertainment and follow-up biases.

The animal studies for oral exposure to heptachlor and heptachlor epoxide are almost all limited to some extent by the number of doses used, the lack of appropriate statistics, or the small number or lack of controls. No information was located regarding the health effects of inhalation or dermal exposure, with the exception of a dermal LD_{50} in rats. Exposure of the general population via the inhalation and dermal routes may result from contaminated soil or vapors from treated houses. Some exposures from contaminated soil or water may occur in populations located near hazardous waste sites in which these chemicals have been stored or from food grown in contaminated soil.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Quantitative methods for the estimation of exposure in humans would be useful. A usable model to estimate the exposure levels from the residue in blood and adipose tissue at various time intervals from the time of exposure would be useful. There are limited data on renal effects from acute exposure of humans to heptachlor or heptachlor epoxide (Derbes et al. 1955). There are a few case reports of neurological and hematological effecfs from occupational or residential inhalation and/or oral exposure to chlordane, a pesticide that typically contains about 10% heptachlor, but there is no way to accurately define the duration of exposure (Dadey and Kammer 1953; Epstein and Ozonoff 1987; Infante et al. 1978). Heptachlor is accumulated in body fat. Acute exposure is likely to result in a delayed effect when the pesticide is subsequently released into the circulation. The liver and the central nervous system appear to be the most sensitive target organs for acute oral toxic effects of heptachlor in animals (Akay and Alp 1981; Aulerich et al. 1990; Kacew and Singhal 1973; Krampl 1971; Lehman 1951,). Although the studies that show hepatic effects are limited (lack of histopathologic examination, enzyme changes that may be adaptive rather than adverse, lack of statistical analyses), the overall pattern of effects indicates that the hepatic function of laboratory animals is altered by acute exposure to heptachlor or heptachlor epoxide.
Acute inhalation studies in animals would be useful for confirming the liver as a target organ by this exposure route, and for providing information about the potential effects on humans exposed in accidents during manufacture or application, or exposed at NPL sites. No acute oral or inhalation MRLs for heptachlor or heptachlor epoxide have been determined because of the shortcomings of the existing database. More information on the effects observed in different species after acute exposure at several dose levels would be particularly helpful. There are no data on acute dermal exposures currently available other than LD_{50} values for heptachlor and heptachlor epoxlde. Since there is a risk of exposure to heptachlor and heptachlor epoxide at NPL sites or from direct contact from residential pesticide application, more information on acute dermal exposures would be useful for determining target organs and health effects from exposure via this route.

Intermediate-Duration Exposure. Because the human studies do not report quantitative information on dose or duration, it is not possible to know with certainty whether the combined inhalation and dermal exposures were of intermediate duration. There are intermediate-duration oral exposure data from animal studies that indicate that the liver and the hematologic systems are affected by heptachlor exposure (Enan

et al. 1982, Halacka et al. 1974; Pelican 1971). The liver is probably the more sensitive of the two. No intermediate-duration oral or inhalation MRLs for heptachlor or heptachlor epoxide have been determined because of limitations in the studies, including lack of statistical comparisons, insufficient number of dose levels, no identification of NOAELs, and the description of effects that may be considered adaptive and not adverse.

There are no data on intermediate-duration inhalation or dermal exposures in either humans or animals. Data on intermediate inhalation and dermal exposures would be useful since the inhalation of vapors or direct contact with residual heptachlor from residential pesticide application or at NPL sites may be potential routes of exposure for the general population.

Chronic-Duration Exposure and Cancer. There are no data on chronic oral exposures in humans. There are occupational studies of workers engaged in the manufacture of heptachlor in which the exposures are presumed to be predominantly inhalation with contributions from the dermal route. No adverse health effects have been identified in these cohorts that could be positively associated with heptachlor exposure (Infante et al. 1978; MacMahon et al. 1988; Stehr-Green et al. 1988). The liver appears to be the most sensitive target organ for the chronic oral toxic effects of heptachlor in animals (University of Cincinnati 1958). Chronic inhalation studies in animals would be useful for determining whether the target organ is the same for both oral and inhalation exposures. There are human case reports that describe neurotoxic and hematologic effects following chronic exposure to technical-grade chlordane from oral or other unspecified routes. Chronic animal studies would be useful for confirming these target organs.

There are occupational mortality studies that have collected data appropriate for determining whether those engaged in the manufacture or application of heptachlor are at increased risk for dying of cancer. These studies have not shown an increased risk of cancer mortality (Infante et al. 1978; MacMahon et al. 1988). Occupational studies that collected cancer incidence data, rather than just mortality data, would be useful for further exploration of this issue.

Other data available for assessing carcinogenicity come from animal studies of rats and three strains of mice (NC1 1977; Williams and Numoto 1984; Witherup et al. 1955). These data show increases in tumorigenesis following exposure to heptachlor. Chronic studies of inhalation exposure in relation to oncogenesis in animals might be useful for determining mechanism of action and the consistency of effect across routes of exposure. There are no pharmacokinetic data that indicate that there will be route-specific differences. There are some data that indicate that male dogs may be more susceptible than females, and female rats store greater amounts of heptachlor epoxide in the liver than do males. Studies that address gender differences would be useful for determining whether these differences may occur in other species.

Genotoxicity. There is very little information on the in vivo genotoxic effects of heptachlor or heptachlor epoxide. This is true for both humans and animals. More case reports and epidemiology studies are needed to properly evaluate genotoxic effects in humans exposed to heptachlor or heptachlor epoxide. In addition, in vivo animal research into the effects of heptachlor and heptachlor epoxide on sister chromatid exchange, chromosomal aberrations and anomalies, DNA adduct formation, gene mutation, and other genotoxic parameters would be helpful in assessing the genotoxic potential of these chemicals. More information is also needed concerning relevant routes of exposure, especially the inhalation and dermal routes.

Reproductive Toxicity. No adverse effects on human reproduction were reported following ingestion of heptachlor-contaminated milk for 27-29 months by women of child-bearing age (Le Marchand et al. 1986).

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Given the uncertain exposure data and the relatively short observation period (relative to human conception and prenatal development), a clear assessment of the relationship between heptachlor exposure and human reproductive toxicity cannot be made. Studies in rodents orally exposed to heptachlor are inconsistent. No adverse effects in mice were reported for acute heptachlor exposure. In an intermediate oral exposure study (60 days), increases in the number of resorptions were seen in the first generation (Green 1970). In the second generation, all females receiving heptachlor at 0.25 mg./kg/day failed to become pregnant. No adverse effects on reproductive capacity were seen in a dominant lethal assay in which eight male mice were exposed to a single oral dose of 25% heptachlor/75% heptachlor epoxide (Arnold et al. 1977). Because the human data do not adequately assess reproductive toxicity and the animal data are inconclusive, additional animal studies evaluating female reproductive end points would be useful for assessing this health effect for all three routes of exposure.

Developmental Toxicity. There are no conclusive data on developmental effects of heptachlor or heptachlor epoxide exposure in humans. Case reports exist that indicate that no adverse developmental effects occurred in the offspring of women who drank heptachlor-contaminated cow's milk (see discussion above on reproductive toxicity). However, heptachlor epoxide has been found in the blood and several tissues of stillborn human infants (Stehr-Green et al. 1986). The identification of heptachlor in amniotic fluid, placenta, and fetal blood provides good evidence of transplacental transfer of this chemical. The relationship of these measurements to exposure is unclear; no data exist that indicate a causal effect. A 60-day oral study in rats showed decreased postnatal survival, but no teratogenic effects were noted (Green 1970). Reproductive studies in rats yielded offspring that developed cataracts at 2-3 weeks after birth, but cataracts also developed in the exposed adults (Mestitzova 1967). Verification of these findings would be useful. Studies that examined both reproductive and developmental effects after intermediate oral or inhalation exposures would be useful because they 'would provide better evidence for establishing the developmental risks in humans.

Immunotoxicity. No studies were located that specifically addressed immune function parameters following heptachlor or heptachlor epoxide exposure. Intermediate and chronic multichemical exposures of humans by inhalation to heptachlor, chlordane, and other substances have been associated with hematologic effects, including aplastic anemia, hemolytic anemia, and leukemia (Epstein and Ozonoff 1987; Infante et al. 1978). The only animal data come from intermediate oral exposure studies in which rats showed a significant elevation of the white blood count (Enan et al. 1982). Rats fed heptachlor for 10 weeks showed increased red blood cells and eosinophils (Enan et al. 1982). Alterations of the hematopoietic system observed following intermediate or chronic multiple chemical exposure suggest that there is at least potential for effects on the immune system. Ninety-day studies examining immune function end points would be useful in establishing whether or not the immune system is a target for heptachlor or heptachlor epoxide toxicity.

Neurotoxicity. The only human data on neurotoxicity come from case reports of occupational exposures to chlordane in which the route was not specified, and for which the effects could not be related directly to heptachlor or heptachlor epoxide alone (Dadey and Kammer 1953). Signs of neurotoxicity, such as irritability, salivation, lethargy, dizziness, labored respiration, muscle tremors, and convulsions, were reported. No data exist describing neurologic effects in animals following inhalation exposure of any duration. Acute and intermediate oral studies in animals provide support for the supposition that the neurotoxicity of chlordane seen in humans may be due in part to heptachlor or heptachlor epoxide. Although there are no reasons to suspect that neurotoxic effects are route-specific, more quantitative data on inhalation effects would be useful because there are no inhalation data and people are exposed by this route in pesticide-treated houses and at NPL sites.

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Epidemiological and Human Dosimetry Studies. The existing epidemiological studies are primarily of occupational cohorts or case reports of health effects seen in groups exposed to contaminated milk (Chadduck et al. 1987). These studies have generally not included good quantitation of the exposure to heptachlor or heptachlor epoxide. In many cases, it is not possible to determine the exact identity of the contaminants involved. Although use of this compound has been discontinued, exposure could nevertheless occur through food grown in contaminated soil, through contact with applied residential pesticides, or from hazardous waste sites. Analytical methods are available to determine exposure to heptachlor or heptachlor epoxide (Curley et al. 1969; Klemmer et al. 1977; Radomski et al. 1968). However, no information is available that correlates levels of heptachlor epoxide in tissue with either level or duration of exposure. Occupational exposure levels are likely to be high enough to enable distinction from background levels. However, many epidemiologic4 studies examining outcomes of exposure are limited by the accuracy of determining the exposure status of those individuals who show adverse health effects and those who show none. The precision and reliability of categorizing exposed individuals and non-exposed individuals contribute significantly to the statistical power of a study and greatly assist in accurate estimation of an increased risk. If data on exposure parameters are sparse or show very wide variation, it is difficult to determine what constitutes an exposure. More data on the correlation of tissue levels to exposure parameters would be useful for increasing the power of epidemiological studies to measure statistically significant associations between heptachlor exposure and health effects in cohorts from both occupational or contaminated community environments.

Biomarkers of Exposure and Effect. Exposure to heptachlor and heptachlor epoxide is currently measured by determining the level of these chemicals in the blood or adipose tissue in living organisms (Curley et al. 1969; Klemmer et al. 1977; Radomski et al. 1968). This measure is specific for both heptachlor and heptachlor epoxide. Heptachlor epoxide is also a metabolite of chlordane, and thus its presence is not specific for exposure to heptachlor alone. However, in the absence of stable chlordane residues (e.g., nonachlor and oxychlordane), the heptachlor epoxide would most likely have been derived from heptachlor. Because heptachlor is believed to be converted rapidly in the body to heptachlor epoxide, it is impossible to determine whether the exposure was to one or the other of these two compounds. Heptachlor and heptachlor epoxide accumulate in adipose tissue and are released slowly over long periods of time. Therefore, it is not possible to accurately identify whether the exposure was recent or what the duration of exposure was. However, the ratio of heptachlor epoxide to heptachlor increases over time and therefore may be used as a biomarker of possible exposure to heptachlor. The sensitivity of the methods for identifying these compounds in human tissue appears to be only sufficient to measure background levels of heptachlor epoxide in the population. Additional biomarkers of exposure to heptachlor would be helpful at this time.

There is no clinical disease state unique to heptachlor. A major problem in developing a biomarker of effect for heptachlor or heptachlor epoxide is that human exposures to these compounds have occurred concomitantly with exposures to other chemicals, and it is difficult to attribute the health effects to heptachlor or heptachlor epoxide alone. More data that quantify the biological effects as well as data that distinguish heptachlor and heptachlor epoxide exposures from those of other chemicals would be useful for developing biomarkers of effect for population monitoring. Biomarkers that could indicate the length of time since exposure would also be useful.

Absorption, Distribution, Metabolism, and Excretion. There are very few data available to assess the relative rates of pharmacokinetic parameters with respect to route of exposure for either heptachlor or heptachlor epoxide. There are no human or animal inhalation or dermal studies on absorption, distribution, metabolism, or excretion. The only human data on metabolism come from in vitro studies

using liver microsomes that indicate that, qualitatively, human microsomes metabolize heptachlor to the same end products as do rat microsomes (Tashiro and Matsumura 1978). Oral exposure in members of farm families led to elevated serum levels of heptachlor metabolites (Stehr-Green et al. 1986), indicating that the compound is absorbed through the gastrointestinal tract. Animal studies also suggest that uptake occurs through the gastrointestinal tract following oral dosing; excretion of these doses occurs primarily through the bile duct into the feces (Tashiro and Matsumura 1978). Animal studies also indicate that heptachlor can be absorbed through the skin to acutely toxic doses, but there are no data on distribution, metabolism, or excretion of dermally absorbed doses. Substantial amounts of data would be useful in order to gain a thorough understanding of the pharmacokinetic parameters of these compounds. Heptachlor epoxide is more toxic than heptachlor and has a longer half-life. Additional pharmacokinetic data on absorption of heptachlor epoxide would be helpful.

Comparative Toxicokinetics. There are limited available data with which to compare humans and other animal species. There are, for example, no inhalation studies in humans and one poorly controlled rabbit inhalation experiment. For the dermal route of exposure, the data are limited to only one rat study (Gaines 1969). With oral exposure, however, heptachlor and heptachlor epoxide seem to have essentially the same absorption and distribution properties in both humans and animals. Although there are no human kinetic data and scanty animal data with which a comparison between humans and animals can be made, the oral distribution data in human cadavers and rats suggest that target organs are similar. The single in vitro comparative study that specifically addresses metabolism indicates that the metabolites produced in humans and rats are identical, but the amounts differ (Tashiro and Matsumura 1978). Moreover, the rate of metabolism is not similar in both species. Thus, the rat may not be an appropriate metabolic model for humans. No information was located regarding human excretion of heptachlor or heptachlor epoxide, and only one study in rats was located. Finally, there is a lack of information regarding kinetic changes after prolonged exposure. This kind of information would be useful because most exposures in the general population (e.g., from contaminated food or improperly applied pesticides) are likely to be long-term and low-dose.

Methods for Reducing Toxic Effects. The mechanism by which heptachlor and heptachlor epoxide are absorbed from the gastrointestinal tract is unknown. Current methods for reducing absorption from the gastrointestinal tract involve removing these chemicals from the site of absorption (Haddad and Winchester 1990; HSDB 1992; Morgan 1982; Stutz and Janusz 1988). Additional studies examining the method of absorption would provide valuable information for developing methods that can interfere with gastrointestinal absorption. Numerous studies have examined the distribution of heptachlor and heptachlor epoxide (Barquet et al. 1981; Burns 1974; Curley et al. 1969; Greer et al. 1980; Jonssan et al. 1977; Polishuk et al. 1977b; Rodomski et al. 1968). Additional studies on distribution are not necessary at this time. No established methods exist for reducing body burden of heptachlor and heptachlor epoxide. However, available information suggests that removal of these compounds via biliary-gastrointestinal excretion can be accelerated (Morgan 1982). Reducing enterohepatic recirculation before these chemicals partition to tissues may be effective (Haddad and Winchester 1990; HSDB 1992). Thus, studies examining the effectiveness of repeated doses of activated charcoal or cholestyramine in reducing body burden would be useful. Adipose tissue serves as a major storage repository for both heptachlor and heptachlor epoxide (Barquet et al. 1981; Burns 1974; Greer et al. 1980; Harradine and McDougall 1986). Losing fat can : mobilize the stored compound and increase the rate of its elimination. However, it may temporarily increase the blood levels of heptachlor epoxide. Studies that would examine the benefits of reducing body burden with accompanying fat reduction while balancing against harmful effects from temporary increase in blood level would be useful. Since heptachlor undergoes epoxidation to produce heptachlor epoxide which is more toxic than the parent compound, studies examining drugs that would inhibit the enzyme

catalyzing this conversion would be helpful. Neurotoxicity of heptachlor epoxide is believed to result, at least in part, from interference with GABA receptor function (Yamaguchi et al. 1980). The available data suggest that benzodiazepenes and barbiturates may be useful in mitigating some of the neurological symptoms of heptachlor epoxide (Bronstein and Currance 1988; HSDB 1992; Morgan 1982; Stutz and Janusz 1988). However, additional studies examining the effectiveness of GABAergic function in mitigating heptachlor epoxide's neurologic effects would be useful. The liver also appears to be a major target organ for the toxic effects of heptachlor and heptachlor epoxide in animals (Akay and Alps 1981; Krampl 1971; Pelikan 1971). An understanding of the mechanism of action in the liver may identify new approaches for reducing the toxic effects.

2.9.3 On-going Studies

EPA is currently examining the systemic and organ toxicity of heptachlor at its Health Effects Research Laboratories in Research Triangle Park, North Carolina (NTP 1990). The testing was scheduled for completion in fiscal year 1990.

L.B. Willett and C.P. Hodgson of Ohio State University, in collaboration with the U.S. Department of Agriculture, are currently investigating reproductive, metabolic, and nutritional disorders following heptachlor exposure from contaminated food in cattle (FEDRIP 1990). These investigators will also determine the cellular alterations that can influence reproductive or other homeostatic mechanisms.

J. Worebey and M. Lewis of Rutgers University are currently investigating a relationship between prenatal exposure to organochlorine pesticides and heavy metals and the subsequent behavioral development of the exposed infants (CRIS/USDA 1990). Infants of 18 months of age will be examined. Behavioral assessment will be primarily focused on three areas: (1) habituation and recovery of attention, (2) lateral@, and (3) emotionality and attachment (CRIS/USDA 1990).

F. Matsumura, sponsored by the National Institute of Environmental Health Sciences (NIEHS), plans to study the toxic effects of chlorinated and pyrethroid pesticides primarily on calcium and sodium regulating processes in the nervous system. To examine the interactions of the pesticides with calcium regulating processes, researchers will use synaptosomal preparations from the brains of rats and the central nervous systems of squid. To examine the interactions of the pesticides with sodium regulating processes, they will collect antibodies directed against sodium channel proteins.

J.E. Trosko (Michigan State University) is studying. the inhibitory action of heptachlor and heptachlor epoxide on cell-to-cell communication in conjunction with their cancer promoting activities.